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Mitochondrial DNA reveals historical maternal lineages and a postglacial expansion of the grey seal in European waters

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ABSTRACT: Three different evolutionarily significant units (ESU) can be discerned in the grey seal *Halichoerus grypus*: in the northwest Atlantic, the northeast Atlantic and the Baltic Sea. The northeast Atlantic population has been strongly affected by hunting around the European mainland, where it is only in the last decades that breeding colonies have reappeared. The southernmost settlement of the northeast Atlantic ESU is found in the Molène archipelago, northwest France. We studied polymorphisms of the mitochondrial control region (MCR) in grey seals sampled along the Atlantic coast of France for over a decade. MCR polymorphisms highlighted the existence of 4 major MCR haplogroups. Comparison of MCR sequences with those in the GenBank database yielded highly significant differentiation between grey seals sampled in France and grey seals originating from the North Sea and the north and east of the British Isles, as well as from the Baltic Sea. MCR haplogroups were also identified in Baltic Sea grey seals, with some being in common with our samples, while others were not. The times of separation between the MCR haplogroups were estimated to be between 84 000 and 20 500 years ago, and differentiation between Baltic Sea and French coast haplotypes was estimated to have occurred approximately 8000 years ago. These data, added to positive expansion signals, suggest that MCR haplogroups correspond to ancestral maternal lineages, isolated in different grey seal refugia during the last ice age and prior to the expansion of the species in the North Atlantic and the separation between the East Atlantic and the Baltic Sea ESUs.

KEY WORDS: Grey seals · Genetic diversity · Marine mammals · Mitochondrial control region · Population divergence time

INTRODUCTION

Marked intraspecific genetic structure exists in various marine mammal species (Palsbøll et al. 1995, Hoffman et al. 2009, Alfonsi et al. 2012, Fontaine et al. 2014, Louis et al. 2014). This may be caused by historical or present environmental variation (Fontaine et al. 2010, Jackson et al. 2014, Hobday et al. 2015), food availability, food preference and habitat use (Foote et al. 2011, Louis et al. 2014). Anthropogenic activities also have direct effects on the intraspecific genetic diversity of, for instance, hunted or by-caught marine mammal species (Mendez et al. 2010, Palsbøll et al. 2013,

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Ruegg et al. 2013). Fidelity to particular geographic sites used for breeding, feeding, or as migratory routes also shapes population structure. Natal philopatry (defined as fidelity to birthplace) and/or fidelity to feeding sites are well known in different species of actively swimming large marine vertebrates (Palsbøll et al. 1995, Bowen & Karl 2007, Jorgensen et al. 2010, Baker et al. 2013). Often, females tend to be more philopatric, while males can travel long distances for breeding or foraging, thus leading to higher differences between populations in the maternally inherited mitochondrial DNA (mtDNA) when compared to bi-parentally inherited nuclear DNA (Bowen & Karl 2007, Baker et al. 2013). In some species, males may also exhibit site fidelity (Louis et al. 2014). Female philopatry and male-mediated gene flow are often encountered in pinnipeds. Southern elephant seals *Mirounga leonina* (Fabiani et al. 2003), walruses *Odobenus rosmarus* (Andersen et al. 1998) and harbor seals *Phoca vitulina* (Burg et al. 1999) show greater population structure for mtDNA than for nuclear DNA markers.

The ability of long-distance travel has been demonstrated in the grey seal *Halichoerus grypus* (e.g. Vincent et al. 2005), which is widely distributed in the North Atlantic Ocean and in the Baltic Sea (Härkönen et al. 2007, Klimova et al. 2014). Philopatric behavior has also been reported (Pomeroy et al. 2000, 2001). Three geographically isolated evolutionarily significant units (ESU), presenting differences in body size, breeding periods and DNA polymorphisms, are assumed to exist (1) in the northwestern North Atlantic along the Canada and north USA coasts, (2) in the Baltic Sea and (3) in the eastern part of the North Atlantic from Iceland to the European mainland and British Isles coasts (Bonner 1981, Boskovic et al. 1996, Klimova et al. 2014).

Subfossil remains confirm the occurrence of the grey seal 5000 years ago (ya) along the European mainland and, in particular, in the southernmost part of its range (Brittany coast in northwest France, Pailler et al. 2004, Härkönen et al. 2007). The species declined and almost disappeared because of hunting before the second part of the 20th century along the European mainland (Härkönen et al. 2007). Breeding sites reappeared at the end of the 1970s on the Dutch and German coasts and subsequently in northwest France in the Molène and Sept-Iles archipelagos (Duguy & Hussenot 1991). Today, east Atlantic grey seals can mainly be found along the UK coast and in the Wadden Sea. Breeding colonies can be found along the mainland from the Molène archipelago in the south to the Kola peninsula (Russia) in the north (Vincent et al. 2005, Härkönen et al. 2007).

Previous studies in the Baltic Sea and in neighboring Atlantic waters have highlighted contrasting patterns between nuclear and mitochondrial loci (Allen et al. 1995, Graves et al. 2009, Fietz et al. 2013, Klimova et al. 2014). Both markers suggested genetic exchanges between adjacent colonies in the eastern part of the UK or in the Baltic Sea (Fietz et al. 2013, Klimova et al. 2014). On a larger scale, genetic differentiation has been observed between geographically distant colonies (e.g. the colonies of northwest Scotland and northeast England) with mitochondrial but not with microsatellite markers (Klimova et al. 2014). On the other hand, a larger influence of the habitat type, rather than geographical closeness, has been demonstrated in the genetic diversity of the major histocompatibility complex immune system gene family (Cammen et al. 2011). All these studies supported a major influence of the fidelity to pupping sites in the shaping of grey seal populations.

In northwest France, satellite-tracking studies have demonstrated grey seal movements from the Iroise Sea to the British colonies, and several authors have therefore suggested the existence of a meta-population at the scale of an area extending from the west coast of France to the southwest British Isles (Vincent et al. 2002, 2005, Gerondeau et al. 2007, Härkönen et al. 2007).

Here, we genetically analyzed a sample of 222 individuals collected by the seal care center of Océanopolis (Brest, France) and by the French Stranding Network (Pelagis, UMS 3462, CNRS, Université de la Rochelle, France) since the 2000s (see Fig. 1). We performed the first genetic study of the grey seal around the coast of Brittany in northwest France, with a special emphasis on maternally inherited mtDNA. We then placed this French population in a wider geographic context, including previously published data from other European and Baltic colonies (Graves et al. 2009, Fietz et al. 2013, Klimova et al. 2014, van Bleijswijk et al. 2014).

MATERIALS AND METHODS

Specimen and sample collections

This study used samples from 222 grey seals. Of these, blood samples were obtained from a total of 168 juvenile grey seals stranded alive along French coasts between 2002 and 2012, which were all taken

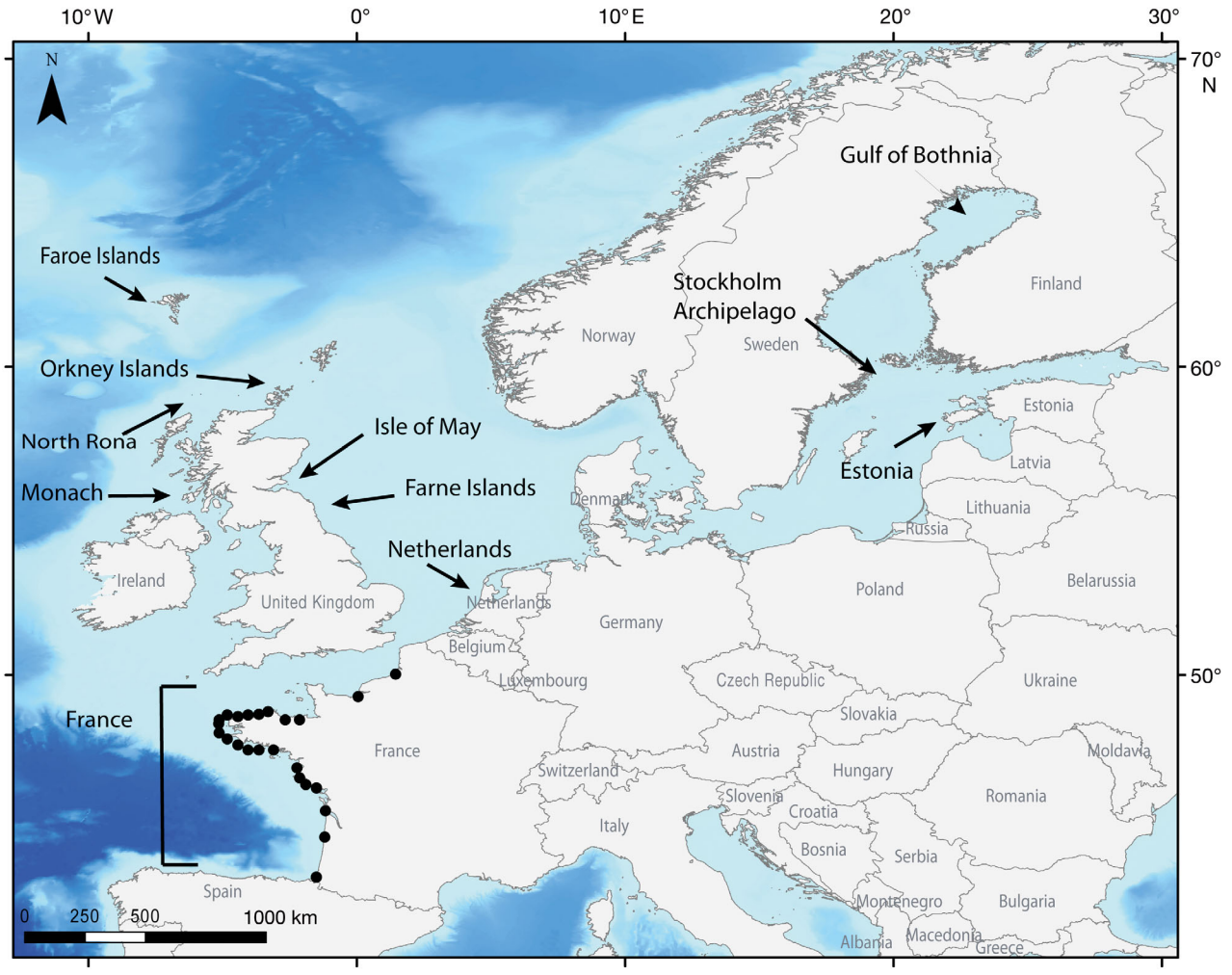

Fig. 1. Location of sampled stranded grey seals on the French coast (black dots) and other European colonies (arrows) compared in this study

in and nursed by the Laboratoire d'Etude des Mammifères Marins (LEMM) at a care center localized at Océanopolis, Brest, France (Fig. 1; Table S1 in the Supplement at www.int-res.com/articles/suppl/m566p217_supp.pdf). For each grey seal, blood samples were collected for veterinary use and subsamples were kept at −20°C for later genetic analyses (Table S1). Samples were named 'Hgc' followed by a number corresponding to a care center identification number (Hgc 227 to 460 in ascending chronological order but not necessarily successive). Additionally, 54 dead stranded grey seals (juveniles and adults) were biopsied in the framework of the French Stranding Network (described in Jung et al. 2009), and skin and muscle samples were used for this study (Table S1). These samples were called 'Hgs' followed by a number.

DNA extraction, PCR amplification and sequencing of MCRs

Total DNA was extracted from blood and tissue samples using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's recommendations. Purified DNA was eluted in Tris HCl-EDTA buffer (pH 7.5) and stored at −20°C. The quality of the extracted DNA was checked by agarose gel electrophoresis, and DNA concentrations were determined using a NanoDrop 100 spectrophotometer (Thermo Scientific). Pinniped-specific primers LMCRHgem and HMCRHgem were used to amplify a 692 bp fragment of the mitochondrial control region (MCR) using the conditions described in Alfonsi et al. (2013). PCR products were purified using the MinElute PCR Purification Kit (Qiagen) and sequenced (GATC Biotech) in the pres-

Table 1. GenBank references of grey seal MCR sequences used for comparisons at the north European scale by geographic localization (Dataset_2). For the east Atlantic and Baltic Sea, 'colonies' are defined according to Klimova et al. (2014). All Orkney Islands colonies (13; Klimova et al. 2014) were grouped as 1 colony

Colonies or region	No. of haplotypes	Length (bp)	GenBank accession nos.	Total no. of specimens	No. of haplotypes (l = 317 bp)	References
French Atlantic coasts						
Northwest France (Brittany)	34	493	KT247900 to –933	222	33	This study
North Sea						
Netherlands	14	374	KM053398, –399, –402, KM066993, –995, –998, –003, –012, –017, –023, –027, –059, –067, –081	98	13	van Bleijswijk et al. (2014)
Northeast Atlantic						
Monach	134	350	KJ769328 to –461	28	15	Klimova et al. (2014)
North Rona				48	15	
Orkney Islands				1245	104	
Isle of May				54	11	
Farne Islands				49	15	
Faroe Islands				31	11	
Baltic Sea						
Estonia	38	435	KF483184 to –221	36	21	Fietz et al. (2013), Klimova et al. (2014)
Stockholm Archipelago				28	14	
Gulf of Bothnia				39	24	

ence of one of the PCR primers. Each haplotype was sequenced at least once in both directions. The GenBank accession numbers are from KT247900 to KT247933 (Table S2 in the Supplement).

Sequence analyses

Sequences were manually edited and aligned with Geneious Pro v.7.1 (Biomatters; Kearse et al. 2012) to produce Dataset_1. To estimate saturation of the MCR, comparison of uncorrected pairwise genetic distances and model-corrected genetic distance (Tamura-Nei [TrN]; see 'Bayesian analysis of population divergence' for model choice) was used as proposed by Philippe et al. (1994) on our total MCR dataset.

All sequences were compared to those available in GenBank using BLAST (Altschul et al. 1997). A total of 186 haplotypes of 350–435 bp in length (Table 1) from grey seals sampled from 10 different colonies of the northeast Atlantic (Monach Islands, North Rona, Orkney Islands, Isle of May, Farne Islands and Faroe Islands, described in Klimova et al. 2014), North Sea (Netherlands; van Bleijswijk et al. 2014) and the Baltic Sea (Estonia, Stockholm Archipelago and Gulf of Bothnia; Fietz et al. 2013) were discernible (Fig. 1). This Dataset_2 had a 317 bp common region and was composed of 276 haplotypes (Table 1). As Klimova et al. (2014) made available the sequence data per specimen, we were able to calculate genetic differentiation indices.

We also constructed Dataset_3 by grouping our sequences plus those from the Baltic Sea. All these haplotypes overlapped on a 399 bp fragment.

Data analyses and mitochondrial haplogroup characterization

For each dataset, the number of haplotypes (Nh), number of polymorphic sites (k), haplotype diversity (*H*) and nucleotide diversity (π) were determined using DnaSP 5.10 software (Librado & Rozas 2009). This program was also used to estimate Fu's *Fs* (Fu 1996) and Tajima's *D* (Tajima 1989) to assess changes in population sizes such as expansions or bottlenecks. To analyze and illustrate the relationships among haplotypes, median-joining networks were constructed with the number of mutations as distances using Network 4.6 software (www.fluxus-engineering.com).

For Dataset_1 (French samples), mitochondrial haplogroups were first detected on the haplotype network and confirmed using (1) a nonmetric multidimensional scaling (nMDS) approach to graphically represent genetic distances among haplotypes, (2) a hierarchical cluster analysis, performed with the free

open-source R v.3.1 (R Core Team 2014), using the Analyses of Phylogenetics and Evolution (APE) and Modern Applied Statistics with Sand (MASS) libraries for distance matrix and nMDS, respectively, and (3) as clades on BEAST trees (see next subsection). For Dataset_3, haplogroups were characterized on the BEAST tree (see next subsection) and on the haplotype network. The haplogroups were also identified on the network constructed with all the available European data (Dataset_2; see Fig. 2).

Possible correlations between mtDNA haplogroups and biological (sex and age of animals), chronological (year of stranding) or geographical (place of stranding) parameters were tested using a χ^2 test.

Genetic differentiation among groups and among colonies was tested using a χ^2 test based on haplotype frequencies (Hudson et al. 1992) and the nearest-neighbor statistic (*Snn*) index based on a sequence distance matrix (Hudson 2000). Random permutations (n = 1000) were used to assess the statistical significance of *Snn* (Hudson 2000).

Bayesian analysis of population divergence

Bayesian estimation of divergence time between mtDNA haplogroups and between French and Baltic Sea MCR haplotypes was done using BEAST v.2.1.3 (Drummond & Bouckaert 2015). The relevant mutation rate for grey seals varies depending on the authors. Boskovic et al. (1996) used a standard rate of mtDNA evolution, probably not appropriate for grey seals, and which can lead to an overestimation of time. Klimova et al. (2014) used a mutation rate of 2.75×10^{-7} substitutions per site per year, evaluated for the MCR of the Steller sea lion (Phillips et al. 2009), and determined times of divergence in better accordance with nuclear DNA-based studies (Graves et al. 2009). No other improved evaluation of the mutation rate, specific to the grey seal, is presently available. A strict clock with a rate of 2.75×10^{-7} substitutions per site per year was therefore imposed.

Akaike's information criterion (AIC) (Akaike 1973), implemented in Modeltest v.3.7 (Posada & Crandall 1998), was used to determine the evolutionary model that best fit the data (TrN + I and TrN + I + G, where G is the shape parameter of the gamma distribution and I is the proportion of invariable sites). Three coalescent models for 3 priors were tested (constant size, expansion and logistic growth). Analyses were performed with 10 million generations, sampling every 1000 generations with 10% discarded as burn-in. Posterior parameter distributions were examined using Tracer v.1.6 to ensure that effective sample site (ESS) values were above 200 for all of them. Bayes factors (BFs) were then calculated in Tracer to ascertain the best-fit population model.

RESULTS

MCR polymorphisms and haplogroup characterization in the French sample

Analysis of the 222 samples (168 Hgc and 54 Hgs) allowed the determination of 222 MCR sequences of 493 bp each. No significant genetic differences were observed between Hgc and Hgs (χ^2 = 32.01, df = 33, p = 0.52; *Snn* = 0.64, p = 0.64). Consequently, all sequences were grouped into Dataset_1 for further analyses. No saturation of the MCR was observed according to the relationships between uncorrected and model-corrected (TrN) genetic distance (slope = 0.97, data not shown). Haplotype and nucleotide diversities were calculated (Table 2), and 34 haplotypes were observed, as defined by 37 polymorphic sites (Table S2 in the Supplement at www.int-res.com/articles/suppl/m566p217_supp.pdf). Values of Tajima's *D* and Fu's *Fs* were both negative, and only Fu's *Fs* was significant (*D* = −0.95, p > 0.10; *Fs* = −11.09, p = 0.008).

The haplotype network captured 4 haplogroups, each of which was separated by at least by 3 mutations, named haplogroups F1, F2, F3 and F4 (Fig. S1 in the Supplement). The nMDS analysis (Fig. S2), a hierarchical cluster analysis (data not shown), and the phylogenic analysis performed using BEAST (see 'Mitochondrial haplogroup divergence time' below), all confirmed the haplogroup separation. No significant correlation was observed between haplogroup members and the age or sex of the animals, nor with the year or the place of stranding (all χ^2 tests with p > 0.05).

Table 2. mtDNA diversity statistics from grey seals stranded alive (juvenile, Hgc) and dead (juveniles and adults, Hgs) in northwest France. n: sample size, l: sequence length (bp), k: no. of polymorphic sites, Nh: no. of haplotypes, *H*: haplotype diversity, π: nucleotide diversity

Samples	n	l	k	Nh	*H*	π
Hgc	168	493	35	31	0.92	0.0081
Hgs	54	493	24	16	0.91	0.0076
Dataset_1 (Hgc and Hgs)	222	493	37	34	0.92	0.0080

Table 3. Pairwise χ^2 and nearest-neighbor statistic (*Snn*) values estimated from MCR polymorphisms among grey seals sampled off the northwest coast of France and other European colonies. All tests were highly significant (France to Monach: $p < 0.05$, France to all other locations: $p < 0.01$)

Statistic	Genetic differentiation between France and:									
	Nether-lands	Monach	North Rona	Orkney Islands	Isle of May	Farne Islands	Faroe Islands	Gulf of Bothania	Estonia	Stockholm Archipelago
χ^2	116.22	54.61	93.53	323.22	82.81	79.94	87.5	245.95	249.86	230.77
Snn	0.7	0.82	0.78	0.78	0.76	0.76	0.84	0.98	0.98	0.97

Larger geographic-scale analysis

The MCR sequences determined during this study (no. of specimens = 222, Nh = 34) and those from north European and Baltic Sea grey seals (no. of specimens = 1656, Nh = 186; Graves et al. 2009, Fietz et al. 2013, Klimova et al. 2014, van Bleijswijk et al. 2014; Table 1) overlapped on a 317 bp fragment. The truncated alignment of 317 bp involved the loss of 1 MCR_Fra haplotype, 2 haplotypes from the Isle of May and 3 haplotypes from the Orkney Islands colonies. This Dataset_2 was composed of 1878 sequences and revealed 155 different haplotypes defined by 85 polymorphic sites, and had large haplotype and nucleotide diversities (H = 0.93, π = 0.012). Values of Tajima's D and Fu's Fs were both negative and highly significant (D = −1.77, $p < 0.01$; Fs = −193.4, $p < 0.001$). Pairwise comparisons of all French MCR sequences with those of other areas provided strongly significant *Snn* values among all pairs (Table 3). The highest *Snn* values were observed between France and the Baltic Sea. The χ^2 tests also revealed significant differences (Table 3). A lower but significant genetic difference was observed between France and the Monach Isles (*Snn* = 0.82, χ^2 = 54.61, $p < 0.05$ for both tests).

The network constructed with Dataset_2, grouping all data available from the Baltic Sea and the northeast Atlantic, allowed the identification of 4 different major haplogroups (named HA, HB, HC and HD), including 6 sub-haplogroups (HA1, HA2, HB1, HB2, HC1,HC2; Fig. 2). The central part of the network was composed of the bulk of the haplotypes from all the regions (Fig. 2). All major haplotypes were shared among several regions, and only 1 haplotype was shared among all regions. Many private haplotypes were observed in the Baltic Sea colonies (n = 34), in the Orkney Islands (n = 77), and in the French region (n = 10). All were minor haplotypes, shared by only a few individuals, except one from France found in 20 samples.

Mitochondrial haplogroup divergence time

Among the 3 coalescent models tested, BF supported the constant size model (log BF > 1.3). In the French sample, haplogroups F2, F3 and F4 were supported by posterior probabilities of 0.99 and 1 (Fig. S3 in the Supplement). Haplogroup F4 was estimated to be the first to diverge, at approximately 43 100 ya (see Table S3 in the Supplement for all 95% posterior interval [PI] values). The divergence time between haplogroups F1 and F2 was estimated to have occurred approximately 26 100 ya, while haplogroup F3 was estimated to have diverged approximately 19 500 ya from haplogroup F1 (Table S3).

The MCR haplotypes determined during this study (n = 34) and the ones deduced from Baltic Sea grey seals (n = 38; Graves et al. 2009, Fietz et al. 2013) overlapped on a 399 bp fragment. This combined dataset (Dataset_3) of n = 72 haplotypes presented a large haplotype diversity (H = 0.99), defined by 47 polymorphic sites, with only 4 shared haplotypes between the 2 locales. Nucleotide diversity was high (π = 0.016), superior to the one of the French-only sample (Table 2). Six haplogroups, observed on the global network as haplogroups or sub-haplogroups (Figs. 2 & 3), were discernible in this new haplotype network (Fig. S4). The majority of the groups included haplotypes from both geographic regions (e.g. HC, HA2, HB1). In contrast, 2 haplogroups contained only Baltic haplotypes (HB2 and HA1), whereas 1 haplogroup was formed only by French haplotypes (HC2).

The haplotypes of all 6 groups and subgroups identified on the network were also clustered on the BEAST tree (Fig. 3). The French haplotypes belonging to haplogroups F2, F3 and F4 were clearly grouped in, respectively, haplogroups HC, HB and HA (Fig. 3).

Haplogroup HC was estimated as being the first to diverge, at approximately 84 000 ya, followed by haplogroups HA and HB at approximately 60 000 ya (see Table S3 for 95% PI values). The sub-haplogroups were individualized approximately between 44 700 and 20 500 ya (Table S3).

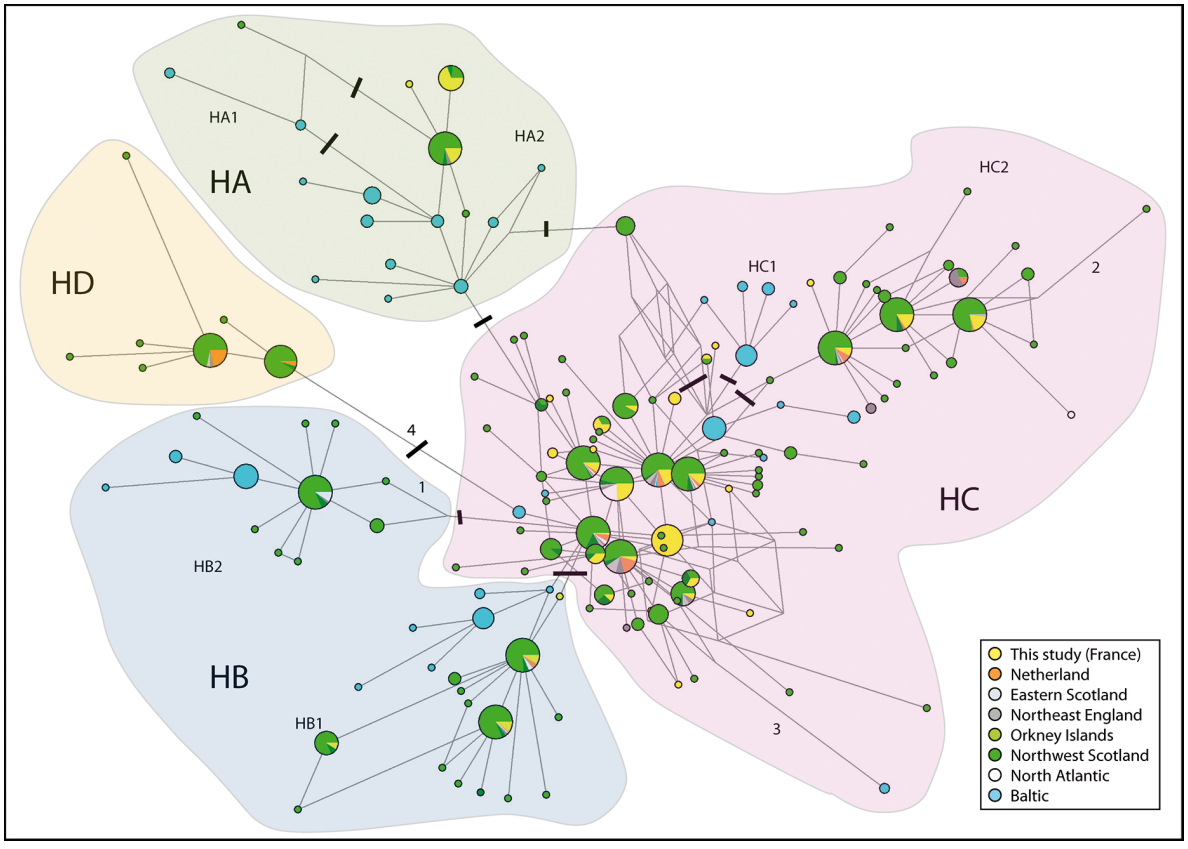

Fig. 2. Haplotype network representation of MCR polymorphisms of grey seal specimens from north Europe and the Baltic Sea (this study, Klimova et al. 2014, van Bleijswijk et al. 2014). All available data (1878 DNA sequences defining 155 haplotypes; Table 1) were used. Numbers above a link indicate number of mutations between haplotypes. The 4 colored areas highlight the 4 haplogroups (HA, HB, HC, HD), and black bars crossing links mark borders between groups and/or subgroups. Colors of the haplotypes represent the geographic regions as defined in Klimova et al. (2014)

In groups HA2 and HB1, groups formed by haplotypes from France have estimated divergence times from the Baltic haplotypes of, respectively, 8500 and 8000 ya (Fig. 3; Table S3).

DISCUSSION

With the exception of newborn whitecoats, grey seals can travel long distances (Stobo et al. 1990, Vincent et al. 2005), and no difference can be made *a priori* between the origin of adults and that of juveniles. Our samples can therefore be considered as representative of a geographic area larger than just the French Atlantic coast. Haplotype and nucleotide diversity were high in our samples, and a genetic structure of 4 MCR haplogroups was discernible. No correlations among these haplogroups and biological, temporal or geographical parameters (age and sex of animals, date and place of stranding) were found, raising questions about their origin.

Comparisons at the European scale

Genetic differentiation indices all highlighted marked differences between French grey seals and those from all other locations. The largest differences were observed between the French and Baltic populations, in agreement with the existence of different ESUs, one in the northeast Atlantic, including the French samples, and another in the Baltic Sea. Other studies also emphasized genetic differentiation, according to distance between colonies, using mitochondrial and/or microsatellite markers (Allen et al. 1995, Boskovic et al. 1996, Graves et al. 2009, Klimova et al. 2014). No DNA sequence data is currently available for grey seals sampled in the south of England, in Ireland or in Wales. Studies including grey seals from these locations will help estimate the actual level of differentiation, and determine whether the British grey seal metapopulation (Gaggiotti et al. 2002) could extend up to the Brittany coasts in France, as hypothesized by Gerondeau et

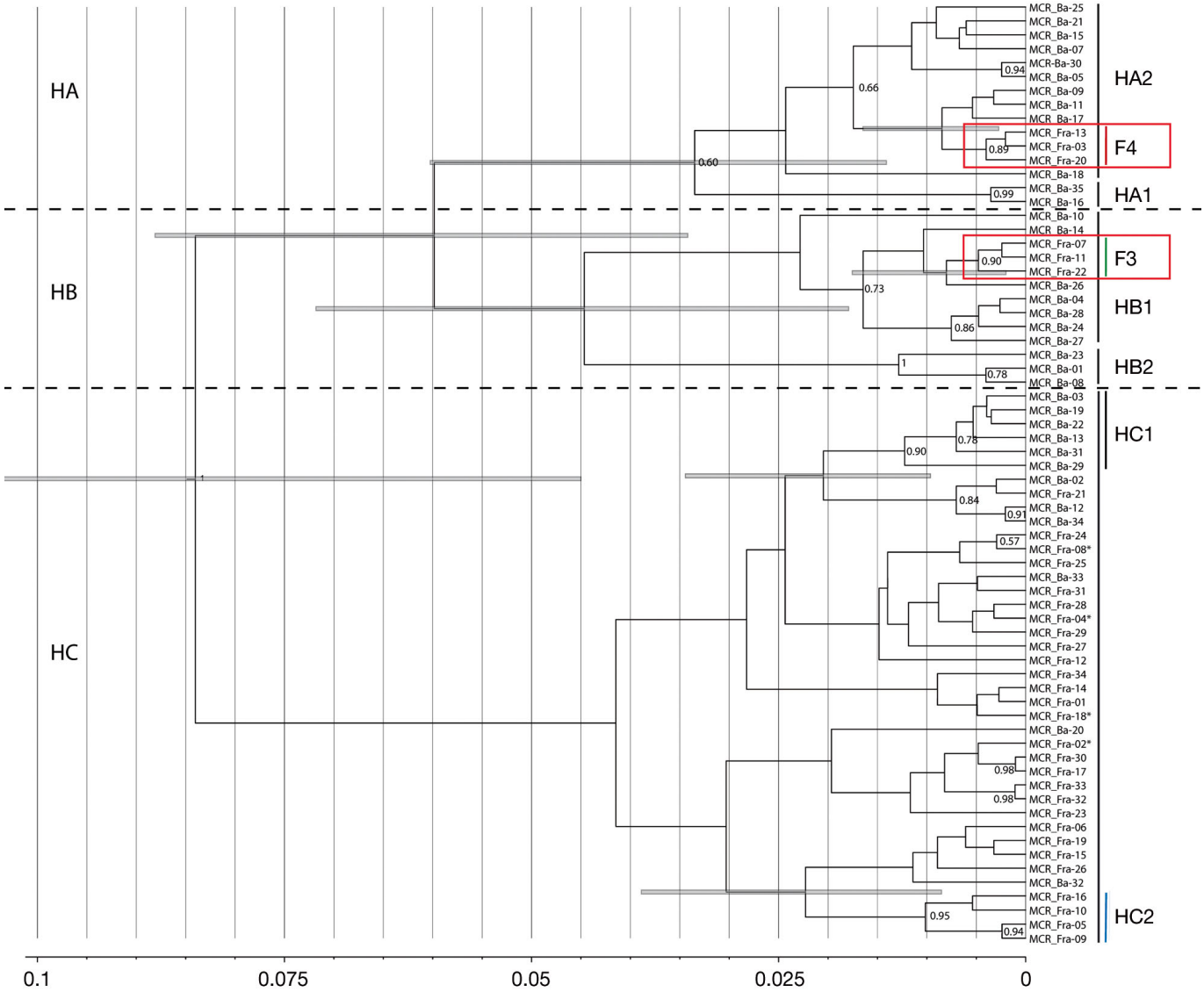

Fig. 3. Bayesian divergence and radiation tree of grey seals from French (this study) and Baltic (Graves et al. 2009, Fietz et al. 2013) coasts based on MCR polymorphisms. Baltic haplotypes were named according to GenBank information. *Haplotypes shared between French and Baltic populations. Posterior probabilities are provided above branches and clades. see Table S3 in the Supplement at www.int-res.com/articles/suppl/m566p217_supp.pdf for divergence time estimates. Grey lines show 95% posterior intervals of key divergence times. The scale axis is in millions of years before the present. Green and red bars show groups formed by French haplotypes F3 and F4 (red boxes) in, respectively, haplogroups HB1 and HA2 and black and blue bars HC1 and HC2, respectively

al. (2007). However, the 10 private haplotypes found in the French samples, as well as the Hudson *Snn* calculation (Hudson 2000), yielded a somewhat high level of genetic differentiation among our samples and those of the north and east British colonies, a result which does not support this hypothesis. Gene flow occurring at a lower geographical scale, among colonies located along the Brittany coast in France, the south of England and Wales, may appear more likely.

The global European network highlights the existence of different haplogroups, some of which are also found in the French samples. It is notable that Baltic haplotypes are distributed in all the haplogroups (with the exception of the sub-haplogroup HC2), suggesting an ancestral origin of our haplogroups older than the separation between the 2 ESUs (between the Baltic Sea and the east Atlantic).

We estimated that the divergence between the Baltic Sea and French coast populations occurred

approximately 8500 ya, a value very close to the estimations made by Klimova et al. (2014) using a different dataset and a different approach (Baltic and northeast Atlantic divergence time estimated between 7000 and 30 000 ya for Klimova et al. 2014, close to the lower estimate of Graves et al. 2009). Klimova et al. (2014) noted that this divergence time could correspond to a transient connection of the Baltic and the North Seas between 10 000 and 9000 ya (Sommer & Benecke 2003). The differences in haplogroup distribution between our samples and those of the Baltic Sea can be explained either by the absence of grey seals of a specific lineage during the colonization of a particular place, or by a more recent loss of the lineage, most likely during the active grey seal hunting periods.

MCR haplogroups and ancestral history in the east Atlantic and the Baltic Sea

Boehme et al. (2012) compared sea surface temperature and bathymetry during the last glacial period to present grey seal habitat preference, estimated from telemetry data. They hypothesized that fewer areas were suitable for the grey seal in the North Atlantic approximately 20 000 ya than at present. A strongly reduced number of grey seals would have occupied refugia, along the Bay of Biscay and Iberian coasts in the east Atlantic, near the Flemish Cap in the west Atlantic, and perhaps in the Mediterranean Sea (Boehme et al. 2012). We hypothesize that these refugia, separated from each other by large areas of unsuitable habitats, have hosted disconnected grey seal populations having no contact or gene flow with each other, and thus becoming genetically distinct.

At the end of the last glacial period, around 12 000 ya, large parts of the coasts of the northwest and northeast Atlantic again became appropriate for grey seals, thus leading to a clear expansion of the species from the different refugia. Such a scenario can explain the present situation, where we detected ancestral lineages older than the geographic separation between the present ESUs. These lineages could well be representative of the different last glacial period refugia.

The positive expansion signals found by Klimova et al. (2014), and also in our study (all the Tajima's *D* and Fu's *Fs* that we determined are negative, and significant for Fu's *Fs*, a more sensitive index to detect recent expansion), strengthen the hypothesis of a grey seal expansion in the North Atlantic. This is in agreement with the fact that other pinniped species have shown positive expansion signals approximately 11 000 ya, at the end of the last glacial age (Westlake & O'Corry-Crowe 2002, Coltman et al. 2007, Dickerson et al. 2010).

Impacts of our results in terms of conservation

For a given species, conservation priorities are often defined at the level of the whole species. Nevertheless, the preservation of intraspecific diversity may be of primary importance (e.g. Baker et al. 2013, Mee et al. 2015). The demographic and genetic characteristics of the 3 grey seal ESUs vary. The interactions with anthropogenic activities and the local protection status contrast greatly between the east and west North Atlantic. Our study focused on a part of the northeast Atlantic grey seal ESU, and our results reinforce previous knowledge of the genetic heterogeneity of this ESU (Allen et al. 1995, Cammen et al. 2011, Klimova et al. 2014). They also highlight the requirement of an increased knowledge at the metapopulation level. Here, we confirmed that the grey seal is clearly a species undergoing a range expansion in the northeast Atlantic since its protection and the end of hunting. But direct and indirect interactions of the seals with fisheries and other threats of anthropogenic origin can have negative impacts (Cronin 2011). The mtDNA groups that we detected during our study should be taken into account in terms of conservation of the grey seal in northwest France and southwest England, as they represent historical maternal lineages whose possible differential presence between British colonies should be further examined.

The Molène archipelago represents the southernmost settlement of the grey seal in the east Atlantic. A particular grey seal diet in this geographic area has been detected by 2 independent studies (Ridoux et al. 2007, Méheust et al. 2015), but whether it reflects opportunistic behavior linked to difference in prey availability or to population specificity has yet to be determined. Further studies to characterize the Molène archipelago grey seals from a genetic point of view, and to determine their possible specificity in a larger metapopulation, are thus warranted.

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