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RESEARCH ARTICLE

# Population structure and historical demography of the thorny skate (*Amblyraja radiata*, Rajidae) in the North Atlantic

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**Abstract** Population genetic structure of the thorny skate (*Amblyraja radiata*) was surveyed in >300 individuals sampled from Newfoundland, Iceland, Norway, the Kattegat and the central North Sea. A 290-bp fragment of the mt cytochrome-b gene was first screened by SSCP. Sequences of SSCP haplotypes revealed 34 haplotypes, 14 of which were unique to Iceland, 3 to Newfoundland, 1 to Norway and 3 to the Kattegat. The global $F_{ST}$ was weak but significant. Removal of the two Kattegat locations, which were the most differentiated, resulted in no significant genetic differentiation. Haplotype diversity was high and evenly distributed across the entire Atlantic ($h = 0.8$) with the exception of the North Sea ($h = 0.48$). Statistical parsimony revealed a star-like genealogy with a central widespread haplotype. A subsequent nested clade analysis led to the inference of contiguous expansion with evidence for long distance dispersal between Newfoundland and Iceland. Historical demographic analysis showed that thorny skates have undergone exponential population expansion that started between 1.1 million and 690,000 years ago; and that the Last Glacial Maximum apparently had little effect. These results strongly differ from those of a parallel study of the thornback ray (*Raja clavata*) in which clear structure and former refugial areas could be identified. Although both species have similar life history traits and overlapping ranges, the continental shelf edge apparently does not present a barrier to migration in *A. radiata*, as it does for *R. clavata*.

**Keywords** *Amblyraja radiata* · Thorny skate · Rajidae · Elasmobranchs · Cytochrome b · Mitochondrial DNA · Population structure · Iceland · Atlantic

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## Introduction

The thorny skate (*Amblyraja radiata*; Rajiformes: Rajidae) is widely distributed throughout the North Atlantic region from Hudson Bay to South Carolina, USA in the west, Greenland, Iceland and Spitzbergen in the north, and from Norway to the southern North Sea (including the western Baltic) in the east. Thorny skates live in shallow coastal waters but extend their habitat to a depth of 1,000 m. Their life cycle is oviparous, producing 20–80 eggs per female each year. There is no passive pelagic larval state; rather, fully formed rays hatch after several months (ca. 8–11 cm disc width) and mature at 5–6 years (Walker 1998). The species seems to be reproductively active all year round in areas where the reproductive biology has been studied (i.e. in the Gulf of Maine Sulikowski et al.

2005, North Sea Walker 1998). Philopatry of rays and skates has also been reported and has indicated relatively small home ranges. Tagging studies in UK waters, for example, have shown that 85% of the individuals remained within a 110 km area (Walker et al. 1997; Dulvy et al. 2000; Heessen 2004). These observations, combined with the life history traits, suggest that population genetic differentiation may be relatively strong in *A. radiata*, as compared with fish characterized by high fecundity, long pelagic larval stages and large migratory ranges. Given that *A. radiata* is characterized by low fecundity, slow growth rate, and late maturity; combined with its vulnerability to over-exploitation (Brander 1981; Heist 1999), an assessment of population genetic structure over its range as compared with other skates/rays is of interest to management and conservation. It is also of interest in the broader phylogeographic and demographic context of historical population growth.

Distributions of the North Atlantic and northern European marine biota have been drastically affected by the Pleistocene glacial/interglacial cycles over the past 2.4 Myr. The last glacial maximum (LGM), which occurred ca. 20,000 years ago, has significantly shaped contemporary distributions of both terrestrial (Hewitt 2000) and shallow-water, marine organisms (Cunningham and Collins 1998). Marine populations either became extinct or were forced to retreat (usually southward) into one or more refugial areas. As the ice retreated, populations expanded and recolonized areas previously covered by ice. Ice-sheet extension also drastically affected sea-level and coastlines; and the Baltic and the North Sea effectively did not exist (Frenzel et al. 1992). Thus, for boreal species, potential refugia are postulated to have occurred at about the latitude of Newfoundland (47°N) in the western Atlantic and Northern Portugal/Spain (43°N) in the eastern Atlantic. In addition, there is some paleoclimatic evidence for a coastal, ice-free zone in southern Iceland (Rundgren and Ingolfsson 1999; Bingham et al. 2003) which is also supported by some genetic studies of benthic marine invertebrates (Wares and Cunningham 2001; Addison and Hart 2005; Govindarajan et al. 2005). The degree to which some demersal skates and rays were affected represents an intermediate case between sessile, benthic organisms and widely foraging pelagic organisms.

Mitochondrial DNA (mtDNA) sequences are appropriate for assessing genetic population structure, phylogeography and in making inferences about underlying historical demographic processes that have shaped present-day structure (Avise 2000). Use of mtDNA has the further advantage that its effective population size ($N_e$) is four times smaller than nuclear DNA $N_e$ due to its haploid nature and generally uniparental inheritance (Birky et al. 1989). Thus, the effect of genetic drift is stronger and a higher level of population differentiation can be observed with mtDNA than with nuclear DNA (e.g., Hoarau et al. 2004). This can be of great importance in populations that do not reach migration/genetic drift equilibrium, as in such areas where recolonization history is recent.

The aim of the present study was to survey regional population genetic and phylogeographic structure in the thorny skate (*A. radiata*) in northern Atlantic. We focused on four questions: (1) to what extent are *A. radiata*'s life history traits a good predictor of population differentiation; (2) how has recent climate history shaped the regional distribution of *A. radiata*; (3) to what extent are historical imprints of refugia, recolonization and demographic expansion detectable; and (4) how do these results compare to those for the thornback ray, *Raja clavata* (Chevolot et al. 2006b)?

## Materials and methods

### Sampling and DNA extraction

A total of 337 rays was sampled from 13 locations during various bottom trawl surveys conducted between 2003 and 2004 (Fig. 1; Table 1). In order to obtain reasonable sample sizes, hauls were pooled in some cases (See Table 1). Pooling rules were guided by tagging studies which suggested that individuals generally remained within a 110-km area. Using a conservative approach, we pooled hauls that were close together, i.e., <50 km apart. At some locations, adults and immature individuals were caught together. In such cases, they were separated to avoid temporal admixture (Waples 1998). These two maturity stages were distinguished based on reproductive criteria, i.e. presence of fully differentiated shell glands for females and fully developed testes and claspers for males (Stehmann 1995). Muscle tissue was collected from each individual and preserved in 70% ethanol. Total genomic DNA was extracted using either a modified CTAB (Hoarau et al. 2002) or a silica-based extraction protocol (Elphinstone et al. 2003).

### SSCP and sequencing

A 290-bp fragment of the cytochrome b (cytb) was amplified by PCR using the primers ArCb-F (5′CACAGATAAAATCCCATTT3′), fluorescently 5′ labeled with 6-FAM and Cb-R (5′CCGCCCAATCACT-

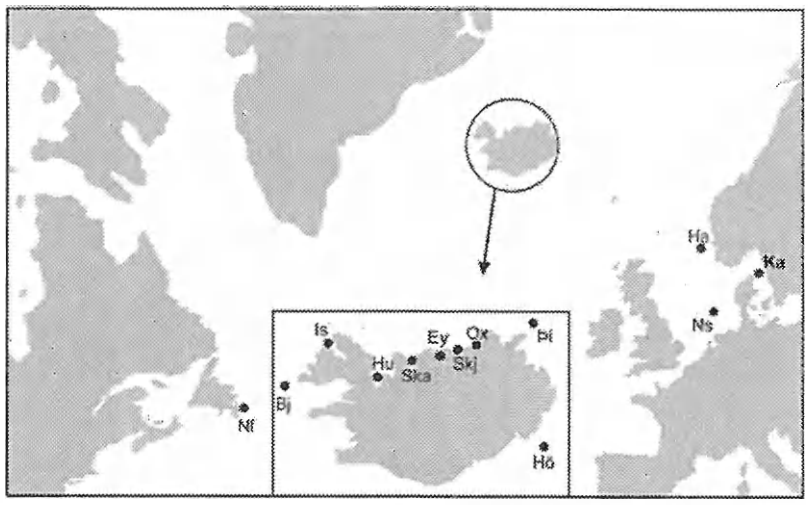


**Fig. 1** Sampling locations for *Amblyraja radiata* (See Table 1 for abbreviations)

CAAACC3'), fluorescently 5' labeled with HEX. PCR reactions were performed in a 10 µl total volume containing 1–3 µl of extracted DNA (<1 ng), 1 × reaction Buffer (Promega), 0.2 mM of each dNTP, 0.25 U *Taq* DNA polymerase (Promega), 2 mM $MgCl_2$, 0.5 µM of ArCb-F and 0.64 µM of Cb-R. PCR amplifications were performed with either a PTC-100™ thermocycler (MJ Research, Inc.) or Mastercycler gradient cycler (Eppendorf). PCR conditions were: initial denaturation for 1 min at 94°C; followed by 32 cycles of: denaturation for 30 s at 94°C, annealing at 49°C for 30 s, and extension at 72°C for 1 min 30, and followed by a final extension step at 72°C for 10 min.

Single strand conformation polymorphism (SSCP) (Orita et al. 1989; Sunnucks et al. 2000) was used to screen for sequence variation in PCR fragments of equal length. Point mutations affect conformation of the single DNA strand, which can be revealed on non-denaturing polyacrylamide gels. SSCP gels were run on an ABI Prism-377 automatic sequencer (Applied Biosystems) as described in Coyer et al. (2002), except that we used 0.3 × MDE concentration and added 5% glycerol to the gel. Because mutations can affect the mobility of one or both strands differently (Lescasse 1999), separate labeling of each strand increases the sensitivity to detect differences.

All gels were analyzed independently and all unique haplotypes detected by SSCP on each gel were sequenced. When more than five individuals showed the same SSCP haplotype, at least two individuals were sequenced. PCR products were cleaned with ExoSapIt (USB Corporation) enzyme following the providers' recommendations. Both strands were sequenced using the Big Dye Terminator Kit (Applied Biosystems) and run on an ABI Prism-377 automatic sequencer (Applied Biosystems). Sequences were edited using BioEdit v7.0.1 (Hall 1999) and aligned with ClustalW and by eye.

## Data analysis

Haplotype ($h$) and nucleotide ($\pi$) diversities (Nei 1987) were estimated using DnaSP v.3.53 (Rozas and Rozas 1999). Population differentiation was assessed using Wright's $F_{ST}$ (Wright 1969). Global and pairwise $F_{ST}$ were estimated using the Weir and Cockerham (1984) $\theta$ estimator with Genetix 4.05 (Belkhir et al. 2004) and significance was tested with 3,000 permutations.

Intraspecific relationships of mtDNA haplotypes were inferred using a statistical parsimony with the software tcs v.1.13 (Clement et al. 2000). The resulting network was used for a Nested Clade Analysis (NCA) to test for geographical association between haplotypes (or nested clades) and geographical distribution (Templeton et al. 1995). The idea is to distinguish between past (fragmentation, expansion) and contemporary processes as tip clades are expected to be more recent than interior clades (Donnelly and Tavare 1986). The first step is to nest the statistical parsimony network following Templeton's nesting rules (Templeton et al. 1995; Templeton and Sing 1993): Haplotypes ("0-step clades") separated by a single mutation are grouped together into a "one-step clade" proceeding from the tips to the interior of the network; then "one-step clades", separated by a single mutation, are grouped together in "two-step clades". The clustering is continued until the next level of nesting would encompass the entire tree. Ambiguities (interconnected haplotypes forming a closed loop due to multiple parsimonious interconnections) were resolved

**Table 1** Sampling locations of *Amblyraja radiata*

| | Sampling site | Code | Latitude (deg) | Longtitude (deg) | $N^a$ | Maturity stage[b] | Sampling date | Cruise[c] | $N_h^d$ | $h^e$ | $\pi^f$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Newfoundland** | Newfoundland | Nf | 46.07 | –47.37 | 30 | A(-A) | October 2004 | DFO Fall survey | 10 | 0.834 | 0.0070 |
| **Iceland** | Off Bjargtangar | Bj | 65.74 | –27.4 | 32 | I(-I) | October 2003 | ICBTS | 9 | 0.802 | 0.0055 |
| | | | 65.67 | –27.56 | 22 | A | October 2003 | | 11 | 0.749 | 0.0060 |
| | | | 65.42 | –27.97 | | | | | | | |
| | | | 65.73 | –28.38 | | | | | | | |
| | | | 65.73 | –27.98 | | | | | | | |
| | | | 65.79 | –27.89 | | | | | | | |
| | | | 65.76 | –27.69 | | | | | | | |
| | | | 65.75 | –27.50 | | | | | | | |
| | | | 65.84 | –27.03 | | | | | | | |
| | Isafjardarjüp | Is | 66.28 | –23.20 | 17 | A | October 2003 | ICBTS | 5 | 0.750 | 0.0030 |
| | Hunafloi | Hu | 65.57 | –21.08 | 27 | A | March2004 | ICBTS | 8 | 0.738 | 0.0047 |
| | Skjalfandi | Skj | 66.14 | –17.54 | 25 | I | March 2004 | ICBTS | 9 | 0.743 | 0.0056 |
| | | | | | 18 | A | | | 9 | 0.824 | 0.0057 |
| | Eyjafjördur | Ey | 66.16 | –18.43 | 19 | A | March 2004 | ICBTS | 6 | 0.784 | 0.0038 |
| | Skagafjördur | Ska | 65.94 | –16.79 | 19 | I | March 2004 | ICBTS | 10 | 0.784 | 0.0058 |
| | Oxafjördur | Ox | 66.28 | –16.28 | 20 | I | March 2004 | ICBTS | 11 | 0.916 | 0.0071 |
| | Off þistlfjördur | þi | 66.91 | –13.48 | 24 | A | October 2003 | ICBTS | 11 | 0.916 | 0.0071 |
| | | | 67.06 | –13.99 | | | | | | | |
| | Off Höfn | Hö | 63.91 | –12.53 | 17 | A | October 2003 | ICBTS | 6 | 0.806 | 0.0036 |
| | | | 63.95 | –12.70 | | | | | | | |
| | | | 64.02 | –12.82 | | | | | | | |
| | | | 64.08 | –12.60 | | | | | | | |
| | | | 64.22 | –12.38 | | | | | | | |
| | | | 63.96 | –12.90 | | | | | | | |
| Eastern Atlantic | Off Haugesund | Ha | 59.37 | 2.92 | 15 | I | February2003 | IBTS | 7 | 0.771 | 0.0039 |
| | | | 58.87 | 3.14 | | | | | | | |
| | Kattegat | Ka | 57.50 | 10.35 | 22 | I | February 2003 | IBTS | 8 | 0.789 | 0.0060 |
| | | | | | 19 | A | | | 6 | 0.719 | 0.0044 |
| | Central North Sea | Ns | 56.13 | 4.81 | 11 | I | February 2003 | IBTS | 3 | 0.473 | 0.0018 |
| **All Locations** | | | | | 337 | | | | 34 | 0.796 | 0.0056 |

[a] Sampling size

[b] A Adults, I Immature

[c] *DFO* Fall survey Department of Fisheries and Oceans, Government of Canada, Fall survey, ICBTS ICelandic Bottom Trawl Survey (Marine Institute of Reykjavik, October and March surveys), *IBTS* International Bottom Trawl survey (International council for the exploitation of the sea)

[d] Number of haplotypes

[e] Haplotype diversity (Nei 1984)

[f] nucleotide diversity (Nei 1984)

following Templeton and Sing's rules (1993). These rules are based on three criteria: the frequency criterion, in which haplotypes are expected to be connected to frequent haplotypes rather than to rare haplotypes; the topology criterion, in which haplotypes are expected to be connected to interior rather than tip haplotypes; and the geographical criterion, in which new haplotypes are expected to remain in the same location as the ancestral haplotype. Using the nested design and the software Geodis 2.4 (Posada et al. 2000), the relationship between haplotype or clades and their geographical distribution was assessed. The program calculates the average clade distances ($D_c$), which is a measure of the geographical spread of a clade; the nested clade distances ($D_n$), which is a measure of how a clade is distributed in comparison to other clades within the same nested clade level; and the interior-tip distances ($I–T_C$ and $I–T_N$) which indicate how widespread younger clades (tips) are compared to their ancestors (interiors), relative to other clades within the same nesting clade. The statistical significance of the distance measures was calculated by comparison with a null distribution after 1,000 permutations. Finally, the biological meaning of the output was interpreted using the latest Templeton inference key (Templeton 2004).

Estimates of past population expansion were made using the mismatch distribution of the cytochrome b sequences (frequencies of pairwise differences between haplotypes), Tajima's $D$ and Fu's estimators and a generalized skyline plot (coalescence approach). Congruence among the three approaches provides stronger inference for population growth or lack thereof. The mismatch analysis assumes that population growth or decline will reveal a genetic signature (i.e., a unimodal distribution) different from that observed with a constant population size (Rogers and Harpending 1992). The observed mismatch distribution was compared to an expected distribution under an expansion growth model and parameters of the model ($\tau$ = time of expansion, $\theta_0$ = population size prior to expansion, $\theta_1$ = final population size) were estimated from the mismatch distribution through the least-square procedure (Schneider and Excoffier 1999). Based on the estimated parameters $\tau$ and the formula $\tau = 2\mu t$, the timing of expansion can be estimated if the substitution rate is known. Substitution rates were estimated at between 0.008 and 0.005 per Myr (Chevolot et al. 2006b). Tajima's $D$ and Fu's estimators test for neutrality, but signature of a population expansion is also given by a significantly negative (Tajima 1989; Fu 1997). These values were estimated using Arlequin v3.0 and significance was tested against 10,000 permutations.

Historical demography was inferred using a coalescent approach through the generalized skyline plot based on the phylogeny of the haplotytpes. The model of variable population size describes the shape of the genealogy depending on the demographic history of the population. This approach is particularly effective for non-resolved, star phylogenies and low sequence variation (Strimmer and Pybus 2001), which is quite often the case for intraspecific phylogenies. The first step in this analysis is to obtain phylogenetic trees from all mtDNA sequences with branch length proportional to time. Therefore, we first calculated the most likely model of molecular evolution to explain the data using ModelTest v3.06 (Posada and Crandall 1998). A hierarchical test of likelihood was performed under 56 models and using the likelihood ratio test (LRT), the HKY model with a gamma rate was selected ($f$(A) = 0.24; $f$(T) = 0.27; $f$(C) = 0.14; $f$(G) = 0.35; transition/transversion ratio = 18.15; gamma shape = 0.015) ($P < 0.0001$). Thereafter, maximum likelihood trees were estimated under the HKY model with gamma shape and with a molecular clock assumption using Paup v4.0b10 (Swofford 1998). The software Genie 3.0 (Pybus et al. 2000) was used to obtain the generalized skyline plot from the maximum likelihood trees with a smoothing algorithm. The ε parameter governs the smoothing algorithm and reduces the noise due to the stochasticity of the coalescence events. This parameter was chosen using the maximize optimization option.

## Results

### Genetic diversity analysis

Among the 337 individuals, 34 haplotypes were detected; differences among haplotypes were due to 26 polymorphic sites, of which 12 were informative. All differences were due to substitutions; no indels were found. The overall haplotype and nucleotide diversities were 0.796 and 0.009, respectively. The highest haplotype diversity was found in Ox, Iceland ($h$ = 0.916), and the lowest in the Central North Sea ($h$ = 0.473). In general, however, haplotype diversity was relatively homogeneous across all other locations (Table 1).

### Population differentiation

Because the global $\theta$ (0.019) was significant ($P$ = 0.001), thorny skates were initially assumed to be significantly differentiated across the sampling sites. Pairwise $\theta$ comparisons showed that most significant pairwise comparisons were due to the two Kattegat locations (Ka-I, Ka-A) (Table 2). Removal of Kattegat populations resulted in a non-significant global $\theta$ (0.008; $P$ = 0.1). No significant genetic differentiation was detected (global $\theta$ = 0.0104, $P$ = 0.12) among all of the Icelandic populations.

### Geographic distribution of haplotypes

The statistical parsimony network among the different haplotypes (Fig. 2) revealed a star-like genealogy with H21 (found in all sampling locations) as the most central and the most frequent (54.5%, Table 3; Fig. 2). The second most common haplotype was H8 (also present in all locations) (Table 3; Fig. 2). Fourteen haplotypes were exclusive to Iceland, three were unique to Newfoundland (H20, H23, H28), one (H32) to Norway, and three to the Kattegat (H7, H10, H16). The nested clade analysis (Fig. 2) revealed 13 one-step clades, 5 two-step clades and the overall network is a three-step clade. A significant association between a clade and its geographic distribution was found in four cases (Table 4). The emerging picture is restricted gene flow between Kattegat, Iceland/Newfoundland (clade 1–3, H7); and the possibility of long-distance dispersal

**Table 2** Population differentiation among the sampling locations based on pairwise multi-locus $\theta$ estimates (Weir and Cockerham 1984).

| Locations | Nf | Bj-I | Bj-A | Is | Hu | Skj-I | Skj-A | Ey | Ska | Ox | pi | Ho | Ha | Ka-I | Ka-A | Ns |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nf | — | 0.005 | 0.019 | 0.006 | 0.012 | 0.011 | 0.009 | 0.022 | 0.022 | 0.016 | 0.19 | 0. 0155 | 0.000 | **0.057*** | **0.056*** | 0.071 |
| Bj-I | | — | 0.031 | −0.016 | −0.005 | −0.014 | **0.025*** | 0.005 | 0.003 | 0.003 | −0.005 | −0.022 | −0.024 | **0.045*** | 0.013 | 0.019 |
| Bj-A | | | — | 0.028 | 0.034 | −0.007 | 0.087 | 0.012 | −0.013 | **0.062*** | −0.018 | 0.043 | −0.015 | **0.053*** | 0.015 | −0.012 |
| Is | | | | — | −0.013 | −0.001 | 0.009 | 0.005 | 0.017 | 0.029 | 0.017 | 0.000 | −0.026 | **0.069*** | **0.060*** | 0.046 |
| Hu | | | | | — | −0.012 | −0.010 | 0.0023 | 0.022 | 0.0182 | 0.0281 | 0.000 | −0.018 | **0.083*** | **0.051*** | 0.039 |
| Skj-I | | | | | | — | 0.035 | −0.004 | −0.013 | 0.030 | −0.004 | 0.000 | −0.029 | **0.057*** | 0.012 | −0.006 |
| Skj-A | | | | | | | — | 0.032 | **0.072*** | −0.007 | 0.060 | 0.021 | 0.016 | **0.101**** | **0.111**** | **0.140*** |
| Ey | | | | | | | | — | −0.010 | 0.030 | 0.003 | 0.027 | −0.026 | 0.05 | 0.022 | 0.044 |
| Ska | | | | | | | | | — | 0.039 | −0.011 | 0.030 | −0.011 | 0.043 | 0.000 | 0.017 |
| Ox | | | | | | | | | | — | 0.021 | −0.014 | 0.017 | **0.066*** | **0.050*** | **0.117*** |
| pi | | | | | | | | | | | — | 0.016 | −0.023 | **0.037*** | 0.009 | 0.015 |
| Ho | | | | | | | | | | | | — | 0.032 | **0.069*** | 0.014 | 0.051 |
| Ha | | | | | | | | | | | | | — | 0.037 | 0.017 | −0.002 |
| Ka-I | | | | | | | | | | | | | | — | 0.021 | **0.111*** |
| Ka-A | | | | | | | | | | | | | | | — | 0.015 |
| Ns | | | | | | | | | | | | | | | | — |

Significant $P$ values are shown in bold

* $P < 0.005$, ** $P < 0.001$

or isolation by distance between Iceland Newfoundland and Kattegat (clade 1–7, H19). Without samples from Greenland and the Faroes, we cannot distinguish between these two scenarios in the last case. Contiguous range expansion is the best explanation for haplotype/clade distribution at the two-step level (clade 2–5, H21) and for the total cladogram, which indicates that there has been high connectivity among locations for a long time.

### Historical demography

The star-like genealogy for the haplotype network is consistent with a sudden exponential expansion of *A. radiata* populations. This is supported by the unimodal mismatch distribution in Fig. 3 and the test of goodness fit of a sudden expansion model that could not be rejected ($P = 0.12$). The estimated parameters of the model were $\tau = 1.414$, $\theta_0 = 0.21$ and $\theta_1 = 1{,}000$, from which the time of expansion for *A. radiata* was estimated to have started between 690,000 and 1,100,000 years ago and not during the LGM (substitution rate = 0.005 and 0.008 per Myr, see Materials and methods). Tajima's $D$ statistics were −1.6 and the Fu's $F_s$ statistics −27.3; both were significant ($P = 0.026$ and $P < 0.001$, respectively). The generalized skyline plot (Fig. 4) is also consistent with past population growth.

## Discussion

### Population differentiation

The near absence of genetic differentiation in *A. radiata* over the North Atlantic does not conform to predictions based on life history characteristics of Rajidae. Although a lack of power related to the small sample size and the use of only one molecular marker might explain this (Waples 1998), the finding of strong and highly significant structure at the ocean basic scale in a parallel study using the same marker for another Rajidae species *Raja clavata* (with a $\theta$ 30 times higher) (Chevolot et al. 2006b) suggests that our results are not an artifact. Both species have similar life history traits but different depth ranges such that, the continental shelf margins are effective barriers.

Tagging studies (mark/recapture) conducted for Rajidae species have indicated traveling distances on the order of hundreds of kilometers, (Templeman 1984; Walker et al. 1997; Hunter et al. 2005) as compared to traveling distances on the order of thousands of kilometers for many bony and cartilaginous fishes

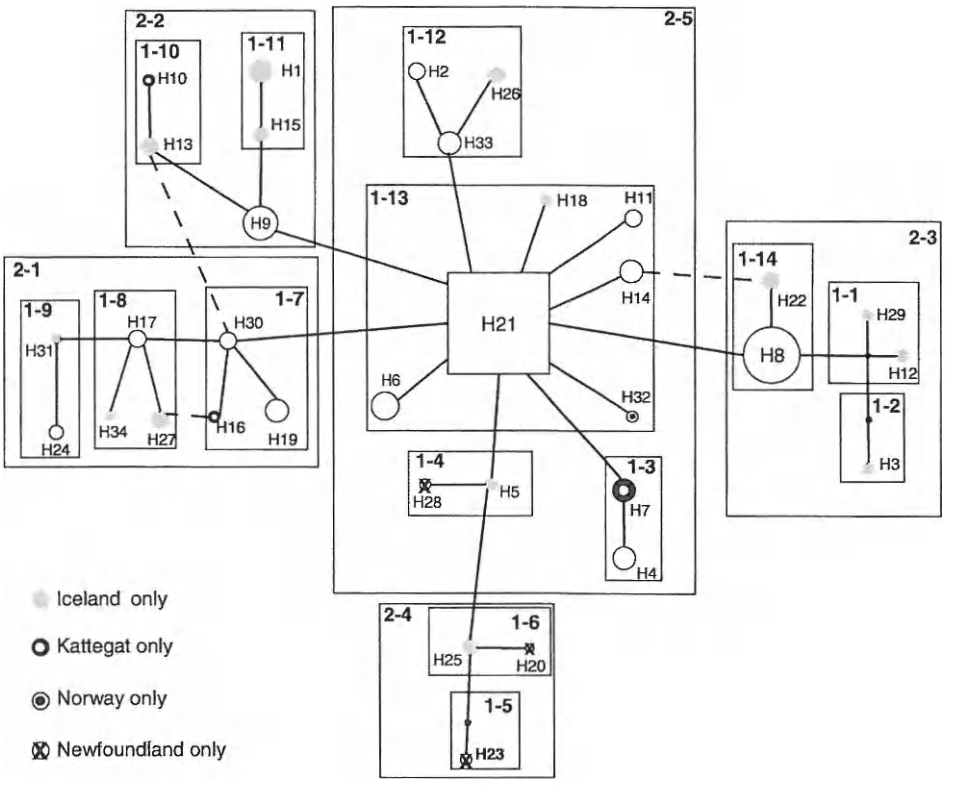


**Fig. 2** Statistical parsimony network (*heavy lines*) and nested clade design (*light lined boxes*) for mtDNA haplotypes of *A. radiata*. The *square* indicates the most probable ancestral haplotype. Other haplotypes are indicated by *circles*; and *small black dots* denote intermediate haplotypes not present in the data set. Each line in the networks between haplotypes represents a single mutational change. Internal ambiguities were resolved following Templeton and Sing (1993), and the less likely paths between haplotypes are shown by a *dashed line*. The nested clade level is given in a hierarchical manner; 1-*n* for 1-step clade, 2-*n* for 2-step clades. The whole cladogram is a 3-step clade

(Metcalfe and Arnold 1997; Lawson and Rose 2000; Kohler and Turner 2001; Sedberry and Loefer 2001; Queiroz et al. 2005) supporting the hypothesis of regional population structure in *A. radiata*. A closer look within the Rajidae, however, reveals that *A. radiata* travels the farthest—between 1.4 and 3 times as far as *R. clavata* (Templeman 1984; Walker et al. 1997). Until now, little attention has been paid to this difference because mismatches between tagging and genetic studies (Hoarau et al. 2004; Chevolot et al. 2006a) have been so great that the variance reported within tagging studies was largely discounted. Tagging studies typically encompass a period of a few years (max 4 years), less than a generation (first age at maturity for *A. radiata* 5–6 years) in Rajidae, whereas genetic studies integrate processes over many generations. Thus, our genetic result on *A. radiata*, suggests that the migratory range is much greater than previously acknowledged. The large scale genetic homogeneity may lie in the fact that thorny skates are not restricted to shallow shelf as they have been caught at depths down to 1,000 m (Stehmann and Bürkel 1994). For example, *A. radiata* is commonly caught across the channel separating Iceland and Greenland continental shelves at approximately 66°N, where depth is as low as 600 m and it is as well regularly fished on the Iceland Faroe Ridge where depth is around 500 m (J. Palsson, Pers. Comm.). This suggests that its wide depth range enables *A. radiata* to migrate over large distances between continental shelves using intercontinental ridges.

The strong differentiation observed between the two Kattegat locations amongst the rest is probably due to differences in salinity and temperature that occur in the transition from the North Sea into the Baltic, as significant restricted gene flow across the transition zone has been shown in several other groups of marine organisms. In the turbot (*Scophthalmus maximus*), no genetic differentiation was found between the Atlantic and the North Sea, but highly significant differentiation was found between the Baltic locations and North Sea/Atlantic locations (Nielsen et al. 2004). Similar results were obtained in the European plaice (*Pleuronectes platesssa*) with the mitochondrial marker (Hoarau et al. 2004) and in the cod (*Gadus morhua*) (Nielsen et al. 2001). Other groups of marine organisms that showed strong differentiation between the North Sea and the Baltic Sea include the seagrass *Zostera marina* (Van Oppen et al. 1995; Reusch et al. 1999; Olsen et al. 2004), the seaweeds *Cladophora rupestris* (Johansson et al. 2003) and *Fucus serratus* (Coyer et al. 2003). Salinity and temperature play a major role in shaping population structure in the herring, *Clupea harengus* (Jørgensen et al. 2005). In all of these cases, local adaptation to environmental conditions may be

**Table 3** Haplotype distributions per sampling sites.

| Haplotype (Accession number) | Nf | Bj-I | Bj-A | Is | Hu | Skj-I | Skj-A | Ey | Ska | Ox | pi | Ho | Ha | Ka-I | Ka-A | Ns | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H1 (DQ 521996) | | 2 | | | 1 | | | | 1 | | | | | | | | 4 |
| H2 (DQ 521997) | 1 | | | | 1 | 1 | 1 | | 1 | 1 | | | | | | | 6 |
| H3 (DQ 521998) | | | | | 1 | | | | | | | | | | | | 1 |
| H4 (DQ 521999) | 2 | 1 | 2 | | | | | | | 1 | 1 | | | 1 | | | 8 |
| H5 (DQ 522000) | | | | | | | | | 1 | | | | | | | | 1 |
| H6 (DQ 522001 | | | | | 2 | 1 | 1 | 3 | 2 | 1 | | | | | 2 | | 12 |
| H7 (DQ 522002) | | | | | | | | | | | | | | 6 | 3 | | 9 |
| H8 (DQ 522003) | 6 | 6 | 2 | 5 | 9 | 5 | 7 | 3 | 1 | 4 | 2 | 4 | 3 | 1 | 1 | 2 | 61 |
| H9 (DQ 522004) | | 3 | | | 1 | 1 | | | | 3 | 1 | 4 | | | 4 | 1 | 18 |
| H10 (DQ 522005) | | | | | | | | | | | | | | | 1 | | 1 |
| H11 (DQ 522006) | | 1 | | 1 | | | | | | | 1 | | 1 | 1 | | | 5 |
| H12 (DQ 522007) | | | | | | 1 | | | | | | | | | | | 1 |
| H13 (DQ 522008) | | | 1 | | | | 1 | | | 1 | 3 | | | | | | 6 |
| H14 (DQ 522009) | | | | 1 | | | | 3 | | | 1 | | 1 | 1 | | | 7 |
| H15 (DQ 522010) | | | 1 | | | 1 | | | 1 | | | | | | | | 3 |
| H16 (DQ 522011) | | | | | | | | | | | | | | 1 | | | 1 |
| H17 (DQ 522012) | | 1 | | | | | 1 | | | 2 | 1 | | 1 | | | | 6 |
| H18 (DQ 522013) | | | | | | | | | | 1 | 1 | | | | | | 2 |
| H19 (DQ 522014) | 5 | | 1 | | | | 1 | | | | | | | | | | 7 |
| H20 (DQ 522015) | 1 | | | | | | | | | | | | | | | | 1 |
| H21 (DQ 522016) | 10 | 13 | 12 | 7 | 11 | 12 | 4 | 8 | 9 | 4 | 11 | 6 | 7 | 7 | 11 | 8 | 140 |
| H22 (DQ 522017) | | 1 | 1 | | | | 1 | 1 | | | | | | | | | 4 |
| H23 (DQ 522018) | 1 | | | | | | | | | | | | | | | | 1 |
| H24 (DQ 522019) | 1 | | | | | | | | | | 1 | 1 | | 1 | | | 4 |
| H25 (DQ 522020) | | | | | | | | | 1 | 1 | 1 | | | | | | 3 |
| H26 (DQ 522021) | | 1 | | | | 1 | | | | | | 1 | | | | | 3 |
| H27 (DQ 522022) | | 1 | 1 | | | | | | | 1 | | | | | | | 3 |
| H28 (DQ 522023) | 2 | | | | | | | | | | | | | | | | 2 |
| H29 (DQ 522024) | | | | | | | | | 1 | | | | | | | | 1 |
| H30 (DQ 522025) | | | | | 1 | | 1 | 1 | | | | | 1 | | | | 4 |
| H31 (DQ 522026) | | | | | | 2 | | | | | | | | | | | 2 |
| H32 (DQ 522027) | | | | | | | | | | | | | 1 | | | | 1 |
| H33 (DQ 522028) | 1 | 2 | | 3 | | | | | 1 | | | 1 | | | | | 8 |
| H34 (DQ 522029) | | | 1 | | | | | | | | | | | | | | 1 |
| Total | 30 | 32 | 22 | 17 | 27 | 25 | 18 | 19 | 19 | 20 | 24 | 17 | 15 | 19 | 22 | 11 | 337 |

important and may lead to stronger genetic differentiation than large geographic distance or depth.

## Phylogeographic patterns

For much of the North Atlantic biota, habitat loss during the LGM resulted in local extinctions and in a generally southward range modifications into periglacial refugia. As ice sheets retreated, populations expanded generally following the leading edge hypothesis (Ibrahim et al. 1996). In this model, theory predicts that the leading edges will be less genetically diverse as founders move away from the genetically-more-diverse former refugia (and under the further assumption of large refugial population sizes). The degree to which this model holds is also expected to differ between shallow benthic and pelagic organisms, the former suffering the most. Rays and skates might be predicted to occupy an intermediate position as they are free to move but are still dependent on bottom substrate for feeding and egg laying.

Low haplotype diversity in the North Sea is consistent with the leading edge model since is has only existed for the past 8,000 years (Zagwijn 1992; Dinter 2001). However, the broad distribution of haplotypes across the entire Atlantic, combined with the star-like haplotype network and the contiguous range expansion does not conform to the usual refugium model but to a high gene flow species with long-term connectivity between the eastern and western Atlantic (Avise et al. 1987; Avise 2000), as it has been found in some teleosts species (e.g. Bremer et al. 2005; Ely et al. 2005). Thus, an East-West Atlantic connection has existed for a long time.

The large number of haplotypes found in Iceland is most likely due to the admixture between western and eastern Atlantic populations as a consequence of its central location rather than as a potential glacial

**Table 4** Phylogeography inference from the Nested clade analysis following Templeton's key (2004)

| Clade | Dominant haplotype/geographical distribution | Inference |
|---|---|---|
| Clade 1–3 | H4/Iceland, Newfoundland and Kattegat | Restricted gene flow |
| Clade 1–7 | H19/Iceland, Newfoundland and Kattegat | Too few samples to distinguish between Long distance dispersal and isolation by distance |
| Clade 2–5 | H21/all sites | Contiguous range expansion |
| Total Cladogram | H21/ all sites | Contiguous range expansion |

Only clade with significant association between haplotype/clades and geographical distribution are shown. See Fig. 2 for the nested design

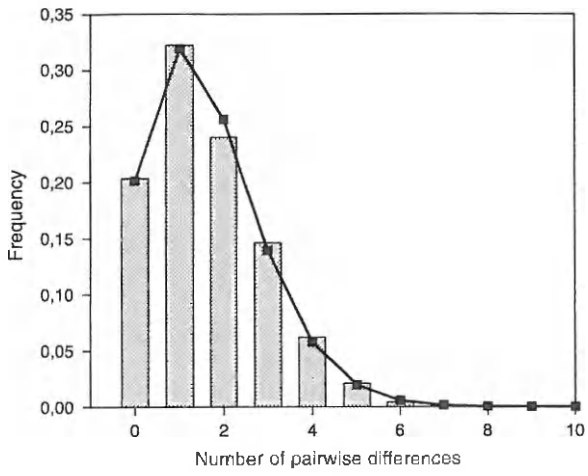


**Fig. 3** Mismatch distribution for *A. radiata*. The *line* represents the expected distribution under a sudden expansion model

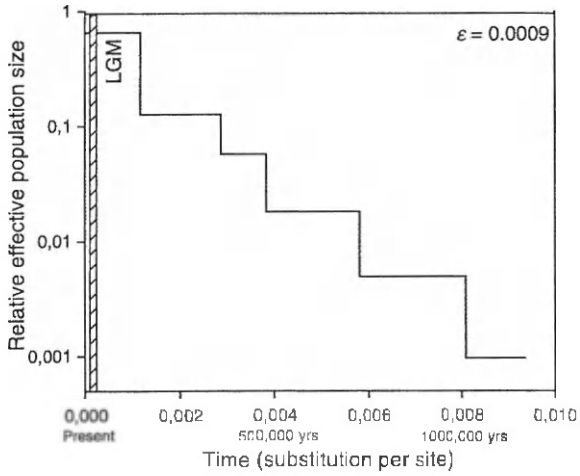


**Fig. 4** Generalized skyline plot of *A. radiata*. The *x*-axis represents the time since the expansion in substitutions per site and in thousands of years; the *y*-axis the estimated effective population size scaled to the substitution rate. The ε-parameter governing the smoothing algorithm was selected from the Akaike Information criterion (AIC). The last glacial maximum period is represented by the *dashed area*

refugium. This is supported by the presence of haplotypes found in Newfoundland and Iceland (H2, H19, H33), and a second set of haplotypes found only in Iceland and Europe (H7, H9, H11, H14, H17, H30). Although our sampling effort was proportionally larger than in the rest of the North Atlantic, most of the Icelandic locations have haplotype diversities within the range of those found in Canada, Norway and the Kattegat.

## Historical demography

Like *R. clavata*, *A. radiata* population expansion definitely predated the end of the LGM and was estimated at 0.6–1.1 Myrs, which corresponds to the Bavelian and Cromerian complexes, both being successions of cold and warm periods (Zagwijn 1992). In the North Atlantic, marine species (so far investigated) seem to follow a pre-LGM expansion model, e.g., the common goby (Gysels et al. 2004), the Atlantic swordfish (Bremer et al. 2005), the Atlantic bluefin tuna (Bremer et al. 2005), Atlantic bigeye tuna (Martinez et al. 2006), the red alga *Palmeria palmata* (Provan et al. 2005), the brown alga *Fucus serratus* (Hoarau et al. submitted), the bivalve *Macoma balthica* (Luttikhuizen et al. 2003) and the estuarine fish, *Ethmalosa fimbriata* (Durand et al. 2005); where the date of expansion was estimated at between 536,000 (for the common goby) and 128,000 years (for the red alga, *Palmeria palmata*). Thus, it is likely that highly mobile species and/or those able to shift in the subtidal fared better in the many glacial-interglacial periods. For *A. radiata*, a general southerly displacement of the distribution probably occurred; population sizes were probably little affected.

To conclude, although *A. radiata* and *R. clavata* share similar life-history traits, different phylogeographic and population genetic structure patterns were found: no significant population differentiation for *A. radiata* in the North Atlantic; and strong population differentiation for *R. clavata* in European waters (Chevolot et al. 2006b). Clearly, life-history traits in

the Rajidae are poor predictors of the population differentiation.

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