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Original article

Genetic diversity of *Borrelia garinii* from *Ixodes uriae* collected in seabird colonies of the northwestern Atlantic Ocean

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ABSTRACT

The occurrence of *Borrelia garinii* in seabird ticks, *Ixodes uriae,* associated with different species of colonial seabirds has been studied since the early 1990s. Research on the population structure of this bacterium in ticks from seabird colonies in the northeastern Atlantic Ocean has revealed admixture between marine and terrestrial tick populations. We studied *B. garinii* genetic diversity and population structure in *I. uriae* collected from seabird colonies in the northwestern Atlantic Ocean, in Newfoundland and Labrador, Canada. We applied a multi-locus sequence typing (MLST) scheme to *B. garinii* found in ticks from four species of seabirds. The *B. garinii* strains found in this seabird colony ecosystem were diverse. Some were very similar to strains from Asia and Europe, including some obtained from human clinical samples, while others formed a divergent group specific to this region of the Atlantic Ocean. Our findings highlight the genetic complexity of *B. garinii* circulating in seabird ticks and their avian hosts but also demonstrate surprisingly close connections between *B. garinii* in this ecosystem and terrestrial sources in Eurasia. Genetic similarities among *B. garinii* from seabird ticks and humans indicate the possibility that *B. garinii* circulating within seabird tick-avian host transmission cycles could directly, or indirectly via connectivity with terrestrial transmission cycles, have consequences for human health.

1. Introduction

Borrelia burgdorferi sensu lato (s.l.) is a bacterial species complex that includes the causative agents of Lyme disease/Lyme borreliosis, the most common vector-borne disease in the Northern Hemisphere. In North America, *B. burgdorferi* sensu stricto (s.s.) is the predominant genospecies known to cause Lyme disease in humans to-date, even though several other members of the species complex have been identified in ticks in the family Ixodidae on the continent (Postic et al., 2007; Margos et al., 2014, 2016; Cutler et al., 2016). *Borrelia burgdorferi* s.l. is transmitted to humans in North America by *Ixodes scapularis* (in eastern, central, and southern regions) and *I. pacificus* (in western, particularly Pacific coastal, areas). In Eurasia, *B. afzelii*, *B. garinii*, *B. bavariensis*, *B. burgdorferi* s.s. and *B. spielmanii* are known to cause Lyme borreliosis in humans (Baranton et al., 1992; Stanek and Strle, 2003; Richter et al., 2004; Margos et al., 2013). The main vectors are *I. ricinus* in western Europe and *I. persulcatus* in eastern Europe and Asia. Reservoir hosts vary among the bacterial genospecies, with *B. afzelii* and *B. bavariensis* associated with rodents, *B. garinii* associated with birds, and *B. burgdorferi* s.s. a generalist for which both birds and rodents are reservoirs (Kurtenbach et al., 2002; Margos et al., 2013).

The transmission cycles of these bacteria, and the risk of human exposure to infected ticks, generally occur in woodland habitats in which ticks can survive during non-parasitic periods of their lifecycle, and where the mammalian and avian hosts for the ticks and bacteria are found (Radolf et al., 2012). However, *B. garinii* was also found in *I. uriae* feeding on Razorbills (*Alca torda*) in the early 1990s in a seabird colony off the coast of Sweden (Olsen et al., 1993). Other seabirds, such as puffins (Gylfe et al., 1999), are now also recognized as reservoirs of this bacterium, and humans may be infected by transmission from *I. uriae*

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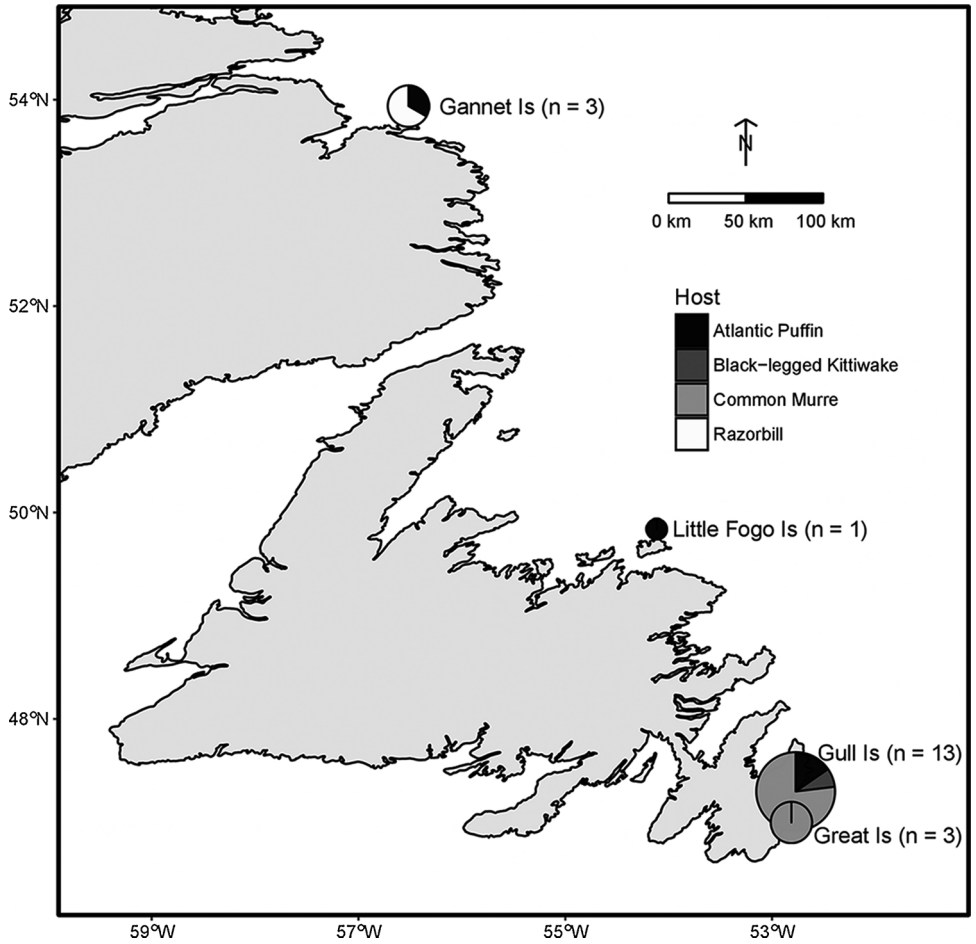

Fig. 1. Geographic locations and avian host species compositions for *Ixodes uriae* sources of *Borrelia garinii* sequences used in this study. The proportions of the different host species are denoted in the pie charts and the numbers of *I. uriae* that contribute to the 20 total ticks analyzed by MLST from each location are in brackets. The map was made using the package "maps" in R (R Core Team, 2017).

(Gylfe et al., 1999). The distribution and prevalence of *B. garinii* in *I. uriae* has now been studied in seabird colonies worldwide, and it has been found associated with a variety of seabird species in both the Northern and Southern Hemispheres (Olsen et al., 1995; Smith et al., 2006; Duneau et al., 2008; Gomez-Diaz et al., 2011).

The circulation of *B. garinii* in the seabird reservoir is complex (Gomez-Diaz et al., 2011), spanning a huge geographic range with many possible avian hosts but *I. uriae* as the only known vector species. *I. uriae* is a seabird specialist that feeds on a range of avian species in multiple families, whereas reports of infestation on mammals are rare (Baggs et al., 2011; Gylfe et al., 1999). More genetic diversity has been found in the marine *I. uriae-B. garinii* system compared to the terrestrial realm involving *I. ricinus* (Comstedt et al., 2009). There is evidence of transhemispheric-scale *B. garinii* movements based on the presence of identical marker gene sequences of the flagellin gene in both the Northern and Southern Hemispheres, shared genetic structure between the Atlantic and Pacific Ocean basins, and little apparent genetic population structure within these ocean basins (Olsen et al., 1995). Recombination analysis has also demonstrated admixture between the terrestrial and marine genetic pools (Olsen et al., 1995) and it is therefore important to study both the marine and terrestrial *B. garinii* cycles to understand circulation of this bacterium.

The genome of *B. burgdorferi* s.l. consists of a linear chromosome, which carries the genes for cell maintenance and replication, and a large number of linear and circular plasmids, which encode most of the outer surface proteins (Osp) that mediate interactions with hosts and vectors (Casjens et al., 2012). Previously, DNA-DNA hybridization and 23S-5S intergenic spacer (IGS) sequences were used to delineate *Borrelia* species (Fukunaga et al., 1995; Baranton et al., 1992). Attempts to classify strains have also utilized 16S-23S IGS sequences (Wang et al., 2014; Bunikis et al., 2004) and the plasmid-encoded *ospA* and *ospC* genes (Qiu et al., 2002; Hellgren et al., 2011). Multi-locus sequence typing (MLST), using core housekeeping genes, has become more widely used (Wang et al., 2014; Margos et al., 2008; Mechai et al., 2015, 2016) as this allows for analysis at multiple genetic levels, from delineation of species (Margos et al., 2009) to exploration of population structure (Mechai et al., 2015).

Newfoundland and Labrador's seabird colonies provide an excellent location to examine patterns of population structure in *B. garinii* as they are tightly connected to colonies in the Canadian Eastern Arctic and sub-Arctic through seabird migration and feeding patterns. Patterns observed in these colonies should also reflect what occurs elsewhere in the Western Atlantic and Arctic seabird colonies. *B. garinii* has previously been documented in three breeding colonies in North America (Smith et al., 2006; Munro et al., 2017), all within the province of Newfoundland and Labrador, but has never been found in the terrestrial realm in North America. Previous studies have documented *B. garinii* in seabird colonies and shown genetic evidence for linkage between strains in terrestrial and marine environments (Dietrich et al., 2011; Gomez-Diaz et al., 2011), but samples from North American seabird colonies have never been included. We have characterized the population structure of *B. garinii* circulating in *I. uriae* within seabird colonies in the northwestern Atlantic Ocean. This was done using an MLST scheme (Margos et al., 2008) that is currently considered the

gold-standard and which has been applied to multiple *Borrelia* species and strains worldwide, allowing comparison of our data to those from *B. garinii* found throughout Eurasia.

2. Methods

2.1. Ethics

Birds were captured and banded under Environment Canada banding permit 10559. This work was carried out under the guidelines specified by the Canadian Council on Animal Care with approved protocols 11-01-AL, 12-01-AL, 13-01-AL, and 14-01-AL from the Memorial University Institutional Animal Care Committee. Lab work was approved under Biosafety Certificate S-103 from the Memorial University Biosafety Committee. Access to the Witless Bay, Gannet Islands, and Cape St. Mary's Ecological Reserves was through permits from the Parks and Natural Areas Division of the Newfoundland and Labrador Department of Environment and Conservation.

2.2. Ixodes uriae collection and Borrelia screening

Between 2011 and 2014, *I. uriae* ticks were collected from four seabird colonies in the northwestern Atlantic Ocean region in Newfoundland and Labrador, Canada (Fig. 1). The Gannet Islands is an archipelago of six islands that features both Common and Thick-billed Murre (*Uria aalge* and *U. lomvia*, respectively), Atlantic Puffin (*Fratercula arctica*), Black-legged Kittiwake (*Rissa tridactyla*), and the highest density of breeding Razorbill in North America. Little Fogo Islands is an archipelago with breeding Atlantic Puffin and Leach's Storm-petrel (*Oceanodroma leucorhoa*). Gull Island is a colony with breeding Herring Gull (*Larus smithsonianus*), Great Black-backed Gull (*L. marinus*), Black-legged Kittiwake, Leach's Storm-petrel, Atlantic Puffin, Common Murre, and Razorbill. Great Island is located 8 km south of Gull Island and hosts a similar assemblage of species. Ticks were collected from the seabird species breeding in each of these colonies that we could readily access.

Ticks were collected directly off birds or from nesting habitat of the seabirds. Ticks collected from the nesting habitat were assumed to be associated with the breeding seabird species from the immediate area. The birds studied at these colonies nest in dense, predominantly single-species, aggregations. Ticks were collected from within nesting material and under rocks less than 30 cm from nest sites. Ticks were also collected from birds that were being captured for other research projects and long-term bird-banding programs. The bodies of birds were examined for ticks with special emphasis on the feet and head as these are areas where *I. uriae* are commonly attached (Eveleigh and Threlfall, 1975; Durden and Keirans, 1996; Cooley and Kohls, 1945) and ticks were removed with fine forceps. Ticks were placed in vials in the field and stored at −20 °C or −80 °C until processed further. All tick life-stages were collected: larva, nymph, and adult (Supplementary Table S1).

DNA was extracted from ticks using the DNeasy Kit (Qiagen). Samples were identified as *Borrelia*-positive using quantitative polymerase chain reaction (qPCR) targeting a conserved portion of the 23S rDNA (Courtney et al., 2004). All samples were screened twice to confirm their positive or negative status. Samples were considered positive if they had a CT value of ≤40 in at least two qPCR assays. In cases where conflicting results were obtained, the qPCR assay was repeated a third time.

Positive samples were subsequently used for attempted PCR amplification of genes used previously for *B. garinii* MLST (Margos et al., 2008): *clpA, clpX, nifS, pepX, pyrG, recG, rplB,* and *uvrA* (Supplementary Table S2). PCR amplifications were performed according to published protocols (Margos et al., 2008) using GoTaq (Promega). All PCR products were sequenced using Sanger sequencing technology at The Center for Applied Genomics (Toronto, Ontario). Sequences were visually examined for ambiguities, primer sequences were removed, forward and reverse sequences were aligned, and consensus sequences trimmed to the lengths of reference sequences using Geneious 8 (Kearse et al., 2012). The possibility of mixed infections, indicated by mixed peaks on sequence chromatograms, was noted and data from such samples were not included in subsequent analyses.

All sequences for the 20 complete MLST profiles were deposited in the NCBI GenBank database with the accession numbers MF536145-MF536294 and added to the pubMLST database (http://pubmlst.org/borrelia/).

2.3. MLST analysis

Sequences from this study were analyzed using the pubMLST database functions for sequence query (http://pubmlst.org/borrelia/) with each allele being ascribed a number corresponding to an existing identical allele, or a new number in the case that the allele sequence was new to the database. Submissions for new allele ID numbers or sequence types (STs) were made to the pubMLST database as appropriate. Based on allelic profiles of 8 housekeeping genes, each sample was assigned an existing or new (for sequences with new combinations of alleles or novel alleles) ST number (Margos et al., 2008; Feil and Enright, 2004). Species accumulation curves were plotted, in R using the package 'vegan' (Oksanen et al., 2017), to examine the increase in ST/allele richness as more samples are considered. The richness of STs was examined relative to geographic locations and host species. Due to uneven sample distribution across geographic locations and host species, and small sample size for some geographic locations and host species, richness was compared between Common Murres and other seabird species at Gull Island and other locations.

2.4. Phylogenetic analysis

To investigate the phylogenetic relationships among *B. garinii* STs, we used those from this study (n = 12) along with those found within the pubMLST database (n = 118), all of which originated from Eurasia. Sequences were aligned using MUSCLE (Edgar, 2004). Model selection was performed using JModelTest (Darriba et al., 2012; Posada, 2008) for each locus and a maximum likelihood tree was produced using PhyML for the concatenated loci (Guindon et al., 2010). Branch support was calculated using a Bayesian-like transformation of the approximate likelihood ratio test (aBayes) because of its high statistical power and calculation speed (Anisimova et al., 2011). Clades with no sequences from seabirds were collapsed for easier viewing, and the tree was rooted with *B. burgdorferi* s.s. as it is in a separate clade (Qiu and Martin, 2014).

2.5. Population structure analysis

Using sequence data from the 12 STs from this study, two different pairwise F_{ST} analyses were performed in R (R Core Team, 2017) using the 'hierfstat' package (Goudet, 2005) to determine the population structure based on colony of sample collection and seabird host. Because we had only one sample from each of Little Fogo Islands and Black-legged Kittiwake, these samples were removed from the analyses. Genetic distance was computed using F_{ST} as previously described (Takezaki and Nei, 1996). To determine the significance of the F_{ST} value, 10000 bootstraps were performed, and the level of significance was altered from $p < 0.05$ by Bonferroni correction to a $p < 0.01$ to account for multiple pairwise comparisons. Genetic distances between populations based on colonies and seabird hosts were determined on this basis.

To identify clonal clustering of our sequences in relationship to all *B. garinii* STs available in the pubMLST database, related clusters of MLST STs were 'classified' into clonal complexes using goeBURST v1.2.1 (Francisco et al., 2009) under the Phyloviz v2 program

Table 1
Sequence types (STs) by colony and tick host.

	Number of ticks	Number of STs	Number of new STs	Number (proportion) of ticks carrying unique STs
Colony				
Gull Island	13	9	2	6 (0.46)
Great Island	3	2	0	1 (0.33)
Little Fogo Islands	1	1	1	0
Gannet Islands	3	3	0	2 (0.67)
Host				
Common Murre	12	9	2	7 (0.58)
Atlantic Puffin	5	4	1	1 (0.20)
Black-legged Kittiwake	1	1	0	0
Razorbill	2	2	0	1 (0.50)

(Francisco et al., 2012). This analysis was performed with all *B. garinii* STs in the pubMLST database as of April 2017. This program is designed for use with MLST data and cluster STs using an algorithm based on a set of hierarchical rules related to the number of single-locus variants (SLVs), double-locus variants (DLVs), and triple-locus variants (TLVs). The goeBURST is the optimized version of the eBURST algorithm and it allows for global optimization and the identification of the founder ST among the set of STs and uses an extended set of tiebreak rules, which leads to improved graphic representation of clonal complexes relating to the ancestral links among ST components. This analysis provides a global perspective of relationships of new and previously described STs, showing founders for the populations and closely related samples based on clonal complexes, as opposed to a phylogeny. Nevertheless, clonal complexes from the MLST analysis and clades on the phylogenetic trees are often concordant (Ogden et al., 2011; Margos et al., 2012; Mechai et al., 2016).

The community structure of the different STs found within the northwestern Atlantic was computed with Bayesian Analysis of Population Structure (BAPS) version 6.0 (Corander and Tang, 2007) using clustering with a linked locus module and codon model. Mixture analysis was performed with K values from 1 to 12, and optimal partitions were identified based on maximum log marginal likelihood values. The analysis was repeated with all *B. garinii* STs in the pubMLST database to identify STs from across Eurasia that clustered with STs found in the northwestern Atlantic, with K values from 2 to 20. This provided an understanding of community structure of the samples from this study and how they fit together on a regional scale, as well as on a larger global scale, and it allowed for clonal complexes to be classified into clusters.

3. Results

3.1. Identification of B. garinii-positive ticks and MLST loci sequencing

Sixty-five out of 865 ticks (7.5%) were identified as *Borrelia*-positive via qPCR screening. These were then used for attempted MLST loci amplifications and sequencing. Mixed sequence chromatogram peaks were observed for three ticks, which were excluded from further analysis. Five of the ticks were determined to be carrying *B. bavariensis* as opposed to *B. garinii* and were excluded from this analysis as these *B. bavariensis* detections are the subject of an independent study (Munro et al., 2017). For the remaining ticks, partial MLST profiles (≤4 of 8 alleles) were obtained for 19, 5–7 of the 8 alleles were obtained for another 9, and full MLST profiles were obtained for 20. All of these sequences, from both complete and incomplete MLST profiles, were confirmed to originate from *B. garinii* by comparison to sequences in the MLST database. The 20 *B. garinii*-positive *I. uriae* for which complete MLST profiles were obtained were collected from four seabird colonies in the northwestern Atlantic Ocean (Fig. 1). Of these ticks, 3 were collected from birds and 17 from the environment, although the majority of ticks from the nesting environment were engorged ticks, i.e. they had recently fed on birds. The strains of *B. garinii* in the ticks from the birds and those from the environment at the same locations would, therefore, be expected to be similar.

3.2. Assignment of B. garinii sequence types

The nucleotide sequences for the eight MLST loci were used to define sequence types (STs). This produced 12 different STs, 10 of which were novel (assigned ST numbers 684 and 686-694). The novel STs contained 26 new alleles and 18 that already existed within the pubMLST database (Supplementary Table S1). These 18 alleles belonged to 31 previously described STs and 65 samples. Only two previously identified STs were found: ST244 and ST575. The richness of neither STs nor alleles in our ticks reached saturation in a species accumulation analysis (Supplementary Figure S1), indicating that increased sampling would result in more unique STs and alleles within this population.

The 12 identified STs were distributed across the four colonies and were identified in ticks associated with four seabird hosts (Table 1). The two previously described STs were found on Gull Island (ST244 and ST575) and Little Fogo Islands (ST575), in ticks collected near (and presumed to have fed on) breeding Common Murre and Atlantic Puffin, respectively. Novel STs were found at all colonies and associated with all seabird species investigated. Novel STs were found in ticks collected directly off birds (two of three ticks) and those collected in the colonies near birds. STs found in the ticks collected from birds were also found in ticks collected from the environment (Supplementary Table S1). The richness (the number of STs per location or seabird host species) did not differ between Gull Island and other locations or between Common Murre and other hosts. The richness also did not differ between ticks collected directly off birds and from the environment. Of the 12 STs identified, unique STs (those not found at another location or associated with another host) were found at each location except for Little Fogo Islands, and were associated with both Common Murre and other seabird hosts (Table 1, Supplementary Table S1, Supplementary Figure S2). The proportion of samples carrying unique STs did not differ between Gull Island and other locations (Fisher exact test, $\chi^2 = 0.016$, $p = 0.90$), or between Common Murre and other seabird hosts (Fisher exact test, $\chi^2 = 0.730$, $p = 0.39$). The relationship between ST richness based on geographic location and host were not independent, with the majority of the samples from Gull Island originating from Common Murre (10 out of 13).

3.3. Phylogenetic relationships of B. garinii from different sources

The sequences found in our study were phylogenetically diverse, with two sequences branching alone and the others falling into three multi-sequence clades (Fig. 2, Supplementary Table S3). Two of these clades, C1 and C4, contained sequences from multiple locations and different host bird species. Each of these clades contained one of the two previously identified STs and clade C4 also contained additional reference sequences from Europe. The third multi-sample clade, C5, contained sequences exclusively from Common Murre on Gull Island and no reference sequences. One of the lone sequences, C2, was basal to clade C1, sharing 99.8% nucleic acid identity with sequences in C1 but differing at every locus with the closest pre-existing ST. The second lone sequence, C3, was basal to a clade of sequences from Europe, with which it shared no alleles at 100% identity.

3.4. Population genetic structure

Pairwise F_{ST} values (Table 2) indicated genetic differentiation and population structuring among localities and tick host species.

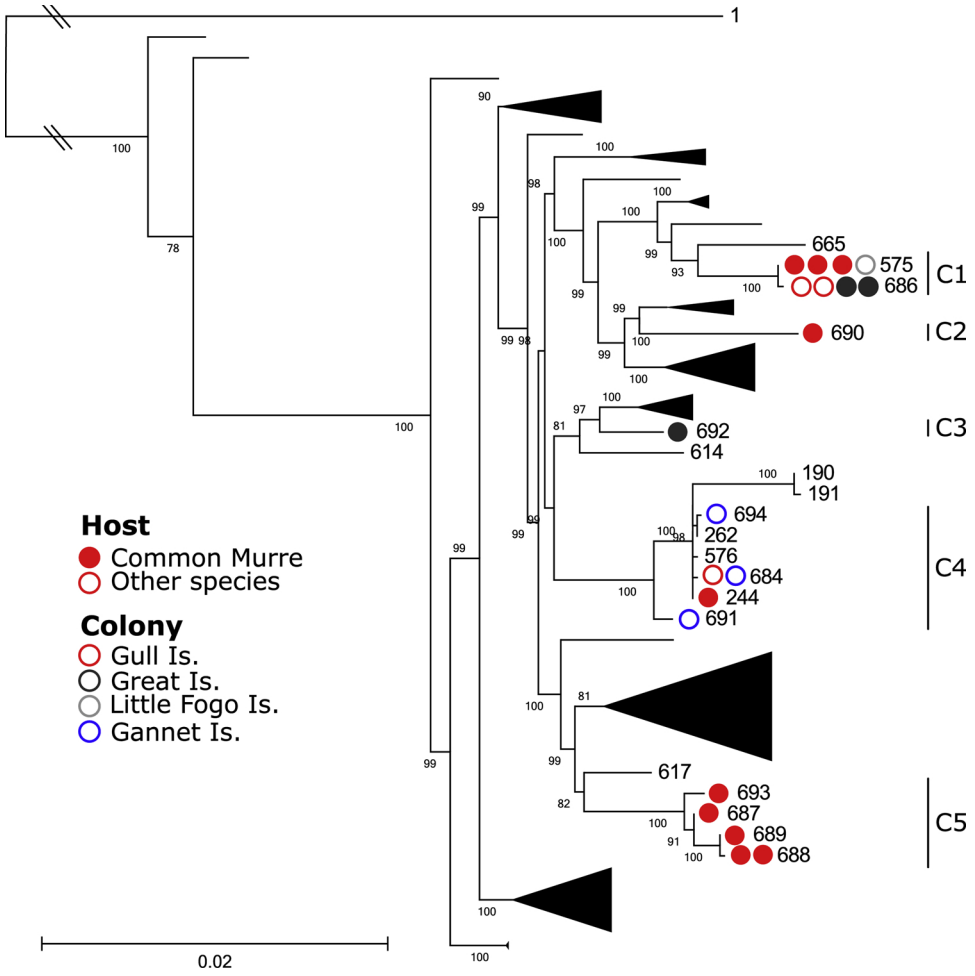

Fig. 2. Phylogenetic analysis of *B. garinii* sequences in *I. uriae* from seabirds in the northwestern Atlantic Ocean. The maximum likelihood phylogeny was constructed using PhyML and the eight concatenated MLST genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB,* and *uvrA*). Labels are sequence types (STs) from the pubMLST database. Sequences from this study are denoted with circles. Colors of the circles indicate colony: red, Gull Island; black, Great Island; grey, Little Fogo Islands; blue, Gannet Islands. Filled circles represent samples from Common Murre and empty circles are all other bird species. *Borrelia burgdorferi* s.s. was used as the outgroup, labeled as "1". Numbers at branch nodes represent support based on aBayes and the scale bar represents the number of substitutions per site. The five branches/clades with sequences from this study are denoted C1 through C5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
Matrix of pairwise F_{ST} values of STs between colonies and bird species (with 99% confidence intervals).

Location	Gannet Islands	Great Island
Great Island	0 (0-0)	
Gull Island	0.05 (0.01-0.08)*	0 (0-0)
Host	**Atlantic Puffin**	**Common Murre**
Common Murre	0.25 (0.11-0.35)*	
Razorbill	0.70 (0.28-1)*	0 (0-0)

* Significant comparisons ($p < 0.01$).

Comparison of STs from Gannet Islands showed significant differentiation from Gull Island ($F_{ST} = 0.049$, $p < 0.01$). There was no differentiation between Great Island and either Gannet Islands or Gull Island. Atlantic Puffin STs showed differentiation from all other species, with the largest value for genetic differentiation being from Razorbill ($F_{ST} = 0.695$, $p < 0.01$). Genetic differentiation varied more among host species than geographic localities/colonies.

We performed a goeBURST analysis with all 130 *B. garinii* STs (118 STs from the database and 12 STs from this study), which revealed that the samples clustered into 21 clonal complexes (using the single-locus variant criterion; SLV) and 63 singletons with eight possible founders (Supplementary Figure S3). However, the 12 STs found in this study clustered into four clonal complexes when either SLVs or both SLVs and double-locus variants (DLVs) were included, including three singletons (Fig. 3, Supplementary Table S3). In this analysis only one clonal complex had an inferred founder, ST244, previously identified in tick and human samples from Germany, Russia, and the UK.

We also performed a Bayesian Analysis of Population Structure (BAPS) on only seabird-origin samples, which suggested the existence of five subpopulations (Fig. 3, Supplementary Table S3) with the highest log marginal likelihood values. These subpopulations showed some geographic structuring, with all STs from the Gannet Islands clustering together with two STs found on Gull Island. There were two subpopulations exclusively from Gull Island, and a single ST found on Great Island formed a solo subpopulation. The final subpopulation contained STs found on Gull Island, Great Island, and Little Fogo Islands. The subpopulations also showed some host structuring, with three subpopulations representing STs only found associated with Common Murre. The remaining two subpopulations contained STs found associated with multiple hosts.

A BAPS analysis using all *B. garinii* STs found in the database supported the existence of three subpopulations, with one containing our samples and the five subpopulations from the northwestern Atlantic Ocean region (Supplementary Figure S3). The subpopulation containing the seabird-origin samples also consisted of samples from across Eurasia and showed no geographic structure. This subpopulation also contained sequences that originated from a range of sources, including various species of ticks and ticks collected from humans.

4. Discussion

In this study, *B. garinii* within *I. uriae* collected from seabird colonies of the northwestern Atlantic Ocean were analysed by MLST. This genetic analysis of *B. garinii* from North America and this ecological system increases the known genetic diversity of *B. garinii* and

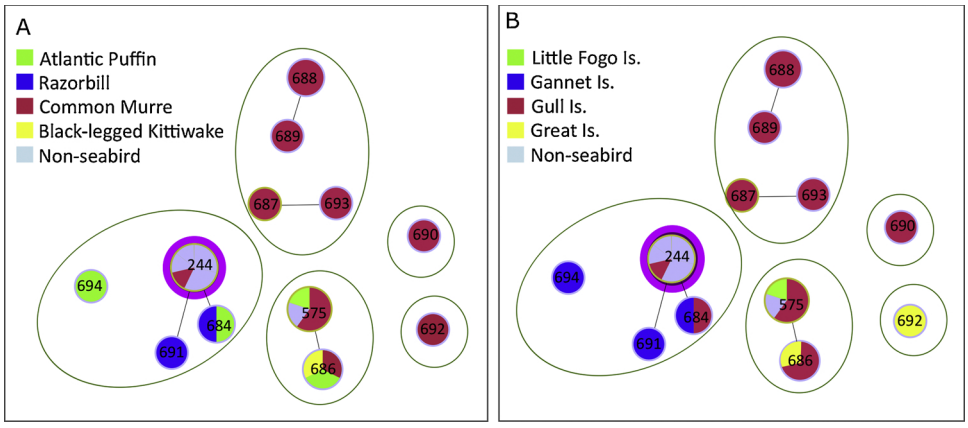

Fig. 3. goeBURST network of the 12 sequences types (STs) of *B. garinii* from this study. The STs are highlighted by seabird host (A) and colony (B) as indicated by the inset legends. The outer dark green circles denote BAPS clusters and the lines denote connections within clonal complexes. The sizes of the circles are proportional to the number of samples in the STs. Inferred founder STs with > 60% bootstrap support are highlighted in pink. Reference sequences originating from sources other than seabirds are indicated in light blue. These analyses considered SLVs and DLVs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

contributes to our understanding of this species globally. We determined that there is population structure in seabird-associated *B. garinii,* at both regional and global scales. At the regional scale and based on our limited dataset of 12 STs, sequences show evidence of genetic clustering by both geographic sites and/or seabird hosts. Sequences found in the northwestern Atlantic region do not all cluster together, which might reflect several independent introductions of the bacterium into this region and/or prolonged circulation with diversification over time. There is also similarity of the northwestern Atlantic sequences to those found in terrestrial ticks and clinical samples from humans in Europe, suggesting connectivity with non-marine *B. garinii* transmission cycles (Gomez-Diaz et al., 2011).

The seabird colonies studied here represent only four of nearly 60 seabird colonies within the Canadian North Atlantic region (Harris and Birkhead, 1985). However, they represent key locations, with the Gannet Islands located in Labrador and closer to Iceland and Europe, where *I. uriae* and *B. garinii* are also present. The more southern locations of Gull and Great Islands, off the eastern coast of Newfoundland, include the largest breeding colonies of Atlantic Puffin in North America and represent a potential link to the colonies further south in Maine and Nova Scotia, where *B. garinii* has yet to be detected (Smith et al., 2006). These colonies are the major coastal seabird colonies (> 100,000 breeding pairs, > 4 breeding species) along the Newfoundland and Labrador shelf. Most of the other colonies are single-species, off-shore, or have fewer breeding birds. Therefore, we believe these colonies represent ideal locations to have studied *B. garinii* in this region.

The prevalence of *Borrelia*-positive ticks found in this study is similar to previous work conducted in the mid-2000s. Research on Gull Island in 2005 and 2006 confirmed the presence of the bacterium in ticks (Smith et al., 2006) at a prevalence of 7.4–20.4% (Muzaffar et al., 2012). The slightly lower prevalence in this study may be a result of seasonal variation, tick-age class composition, and/or seabird/host composition studied.

Although other species of *Borrelia* have been found circulating in the *I. uriae*-seabird system, including *B. burgdorferi* s.s., *B. bavariensis*, and *B. lusitaniae*, *B. garinii* is predominant (Gylfe et al., 1999; Olsen et al., 1995; Dietrich et al., 2011; Gomez-Diaz et al., 2010; Munro et al., 2017). Two STs we found (ST244 and ST575) are identical to STs previously identified in Europe. Indeed, one of these STs, ST244, has a wide geographic range and, in addition to being found in our study, is represented by six samples in the database from the UK, Germany, and Russia, and associated with diverse sources (e.g., human cerebrospinal fluid and *I. persulcatus* and *I. ricinus* ticks). We found this ST in a tick collected from a Common Murre on Gull Island. The other previously described ST, ST575, has only been found in Germany, where it originated from a human skin sample collected in 1994. In addition to these two STs that were found in our samples, 18 alleles were identical those found in the pubMLST databases, which belong to 31 previously described STs and 65 samples. These samples include human clinical samples and positive ticks (*I. persulcatus, I. pavlovskyi* and *I. ricinus*) and originate from seven countries (UK, France, Latvia, Germany, Italy, Norway, and Russia). It is unknown how many of these were from songbirds. These shared alleles demonstrate connections between the strains present in marine birds in the northwest Atlantic and from diverse sources and locations in Eurasia.

The high level of sequence identity for samples from North American and Eurasian sources indicates connectivity of *B. garinii* populations across the North Atlantic Ocean. Furthermore, the documentation of multiple STs in both Eurasia and North America indicate there are frequent movements of the bacterium between these regions. Possible scenarios for movement of the bacterium include transport in infected ticks or in infected birds. Although not impossible, the movement of ticks on seabirds across the Atlantic is unlikely as the period of tick attachment is 4-8 days (Eveleigh and Threlfall, 1974; Barton et al., 1995) while it would take many days to cross the Atlantic Ocean, and land visits by seabird species outside the nesting season along the way are unlikely (Frederiksen et al., 2016, 2012). The seabirds studied here generally leave their colonies at the end of the breeding season and spend most of the rest of the year at sea, with no visits to land before the subsequent breeding season. Therefore, it is more likely that bacteria are moved between colonies in infected birds, especially if the birds remain persistently infected, as is often the case for mammalian hosts (Donahue et al., 1987). There is also experimental evidence for persistent latent infection in a migratory songbird, the redwing thrush (*Turdus iliacus*), lasting at least 3 months after the initial infection (Gylfe et al., 2000). Adult seabirds have high nest-site fidelity but young adults are known to prospect for new breeding locations, resulting in dispersal of birds, and perhaps *B. garinii*, among colonies (Breton et al., 2006; Frederiksen et al., 2016).

High genetic diversity has been documented in past studies of *B. garinii* in *I. uriae* and seabirds (Duneau et al., 2008; Gomez-Diaz et al., 2011; Comstedt et al., 2009) and this was also observed in our data. Twelve STs are present in the 20 ticks analyzed, along with many unique alleles. A similarly high level of richness is also seen in Europe (James et al., 2014) and at multiple geographic levels (Vollmer et al., 2011, 2013). In contrast, a much lower richness is observed in *B. burgdorferi* s.s. in North America, with 111 STs identified in 564 samples, although diversity of *B. burgdorferi* s.s. does differ among geographic regions (Mechai et al., 2013). *Borrelia garinii* is known to be one of the most heterogeneous of the *Borrelia* species, having both high genetic and antigenic diversity (Gern and Humair, 2002). The diversity found in our study is only a small snapshot of what actually exists in these seabird-tick ecosystems and more novel STs are likely to be found with further sampling, as was supported by the species accumulation analysis. Three ticks with evidence of mixed infections were observed, indicating opportunities for recombination among strains. We recognize that removal of these ticks means we potentially did not

identify all strains in our samples, but these were only a small number of the total positive ticks (3 out of 65).

The *B. garinii* found in the northwestern Atlantic region show surprising phylogeographic relationships to sequences collected throughout Eurasia, from non-marine ecosystems and humans. Our sequences are dispersed throughout the *B. garinii* MLST tree, appearing as two singletons and three clusters. Some show close relationships with sequences from throughout Eurasia. This suggests multiple movements of strains and mixing between regions (Bunikis et al., 1996; Comstedt et al., 2009; Gomez-Diaz et al., 2011; Olsen et al., 1995).

At a local level, our data show that a high level of *B. garinii* diversity exists in the northwestern Atlantic seabird colonies, with several independent and divergent clonal groups, consistent with what is found in the eastern Atlantic (Gomez-Diaz et al., 2011). The distribution of genotypes shows some heterogeneity. One cluster of STs (ST693, ST687, ST688, and ST689) originated solely from Common Murres on Gull Island. The other two clusters both comprise multiple ticks and originate from two or more colonies and two or more seabird hosts. Additionally, one cluster consists of STs primarily originating from non-Common Murre hosts, with four such ticks giving rise to three STs (ST694, ST684, and ST691) and a single Common Murre tick containing the other ST (ST244) in this cluster.

There was no relationship between ST diversity and geographic location or tick host based on the phylogenetic and BAPS analyses, which may suggest that there are no processes limiting transmission of the bacterium at either geographic or host levels. However, examination of the genetic distances between populations by F_{ST} analysis did show some level of differentiation. Significant genetic differentiation was observed across large geographic distances, with the Gannet Islands different from Gull Island in Witless Bay. This site is approximately 800 km northwest from Gull Island. Interestingly this pattern does not hold when comparing Gannet Islands with Great Island, which are equi-distant. This pattern may be driven by *I. uriae* population structure, which has been observed among colonies in Iceland and Norway (McCoy et al., 1999; Kempf et al., 2009; Dietrich et al., 2014). Vector-borne pathogens co-occur with their hosts and vectors, and the population genetic structure of hosts and vectors is expected to have a strong driving force on the microbe's structure (McCoy, 2008; Woolhouse et al., 2002). Lack of genetic distance between Gull and Great Islands is not surprising as they are > 7 km apart, share similar seabird species compositions, and would have the easiest opportunities for exchanges of birds, ticks and bacteria.

At the tick host level, genetic differentiation exists between STs found associated with Atlantic Puffin and both Common Murre and Razorbill. The puffins use a distinct breeding habitat, nesting in earthen burrows along grassy slopes (Harris, 1980; Lowther et al., 2002), whereas the other two species are found along rocky cliffs edges (Hatch et al., 2009; Tuck, 1960; Ainley et al., 2002), or talus slopes (Lavers et al., 2009). Therefore, the differences among bird species might be attributable to population structure at the level of *I. uriae* around their seabird hosts on a local geographic level (Dietrich et al., 2013; McCoy et al., 2002) and this could further drive the large geographic patterns seen. Population subdivisions, like those seen among these seabird species, may act as barriers to gene flow for these bacteria and other pathogens (i.e., multiple niche polymorphism (Kurtenbach et al., 2006)).

Overall, this study has contributed to a broader global understanding of *B. garinii* circulation. There is some evidence of host species associations and differentiation across larger geographic distances, but also connectivity among *B. garinii* found in seabird colonies of the northwestern and northeastern Atlantic Ocean and in humans and non-marine ticks of Eurasia. These connections suggest a complicated circulation system with movement across large geographic scales that we propose is linked to bird migration. More research is needed to determine the mechanism(s) connecting the marine and terrestrial ecosystems.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.06.014.

References

Ainley, D.G., Nettleship, D.N., Carter, H.R., Storey, A.E., 2002. Common Murre (*Uria aalge*). In: Rodewald, P.G. (Ed.), The Birds of North America. Cornell Lab of Ornithology, Ithaca, NY. https://doi.org/10.2173/bna.666.

Anisimova, M., Gil, M., Dufayard, J.-F., Dessimoz, C., Gascuel, O., 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Syst. Biol. 60, 685–699. https://doi.org/10.1093/sysbio/syr041.

Baggs, E.M., Stack, S.H., Finney-Crawley, J.R., Simon, N.P.P., 2011. *Peromyscus maniculatus*, a possible reservoir host of *Borrelia garinii* from the Gannet Islands off Newfoundland and Labrador. J. Parasitol. 97, 792–794. https://doi.org/10.1645/GE-2548.1.

Baranton, G., Postic, D., Saint Girons, I., Boerlin, P., Piffaretti, J.C., Assous, M., Grimont, P.A., 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. 42, 378–383. https://doi.org/10.1099/00207713-42-3-378.

Barton, T.R., Harris, M.P., Wanless, S., 1995. Natural attachment duration of nymphs of the tick *Ixodes uriae* (Acari: Ixodidae) on kittiwake *Rissa tridactyla* nestlings. Exp. Appl. Acarol. 19, 499–509. https://doi.org/10.1007/BF00052918.

Breton, A.R., Diamond, A.W., Kress, S.W., 2006. Encounter, survival, and movement probabilities from an Atlantic Puffin (*Fratercula arctica*) metapopulation. Ecol. Monogr. 76, 133–149. https://doi.org/10.1890/05-0704.

Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., Barbour, A.G., 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology 150, 1741–1755. https://doi.org/10.1099/mic.0.26944-0.

Bunikis, J., Olsén, B., Fingerle, V., Bonnedahl, J., Wilske, B., Bergström, S., 1996. Molecular polymorphism of the Lyme disease agent *Borrelia garinii* in northern Europe is influenced by a novel enzootic *Borrelia* focus in the North Atlantic. J. Clin. Microbiol. 34, 364–368.

Casjens, S.R., Mongodin, E.F., Qiu, W.-G., Luft, B.J., Schutzer, S.E., Gilcrease, E.B., Huang, W.M., Vujadinovic, M., Aron, J.K., Vargas, L.C., Freeman, S., Radune, D., Weidman, J.F., Dimitrov, G.I., Khouri, H.M., Sosa, J.E., Halpin, R.A., Dunn, J.J., Fraser, C.M., 2012. Genome stability of Lyme disease spirochetes: comparative genomics of *Borrelia burgdorferi* plasmids. PLoS One 7, e33280. https://doi.org/10.1371/journal.pone.0033280.

Comstedt, P., Asokliene, L., Eliasson, I., Olsen, B., Wallensten, A., Bunikis, J., Bergström, S., 2009. Complex population structure of Lyme borreliosis group spirochete *Borrelia garinii* in subarctic Eurasia. PLoS One 4, e5841. https://doi.org/10.1371/journal.pone.0005841.

Cooley, R.A., Kohls, G.M., 1945. The genus *Ixodes* in North America, National Institute of Health Bulletin. U.S. Public Health Service, Washington.

Corander, J., Tang, J., 2007. Bayesian analysis of population structure based on linked molecular information. Math. Biosci. 205, 19–31. https://doi.org/10.1016/j.mbs.2006.09.015.

Courtney, J.W., Kostelnik, L.M., Zeidner, N.S., Massung, R.F., 2004. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J. Clin. Microbiol. 42, 3164–3168. https://doi.org/10.1128/JCM.42.7.3164-3168.2004.

Cutler, S.J., Ruzic-Sabljic, E., Potkonjak, A., 2016. Emerging borreliae – expanding beyond Lyme borreliosis. Mol. Cell. Probes 31, 22–27. https://doi.org/10.1016/j.mcp.2016.08.003.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772. https://doi.org/10.1038/nmeth.2109.

Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick ixodes uriae. Biol. J. Linn. Soc. Lond. 108, 323–334. https://doi.org/10.1111/j.1095-8312.2012.02021.x.

Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. Vector Borne Zoonotic Dis. 11, 453–470. https://doi.org/10.1089/vbz.2010.0009.

Dietrich, M., Kempf, F., Boulinier, T., McCoy, K.D., 2014. Tracing the colonization and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. Mol. Ecol. 23,

3292–3305. https://doi.org/10.1111/mec.12815.
Donahue, J.G., Piesman, J., Spielman, A., 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hygiene 36, 92–96.
Duneau, D., Boulinier, T., Gomez-Diaz, E., Petersen, A., Tveraa, T., Barrett, R.T., McCoy, K.D., 2008. Prevalence and diversity of Lyme borreliosis bacteria in marine birds. Infect. Genet. Evol. 8, 352–359. https://doi.org/10.1016/j.meegid.2008.02.006.
Durden, L.A., Keirans, J.E., 1996. Nymphs of the Genus *Ixodes* (Acari: Ixodidae) of the United States: Taxonomy, Identification Key, Distribution, Hosts, and Medical/Veterinary Importance. Entomological Society of America, Lanham, MD.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.
Eveleigh, E.S., Threlfall, W., 1975. Bionomics of *Ixodes* (*Ceratixodes*) *uriae* White, 1852 on auks (Alcidae) from Newfoundland. Can. J. Zool. 53, 82–86.
Eveleigh, E.S., Threlfall, W., 1974. The biology of *Ixodes* (*Ceratixodes*) *uriae* White, 1852 in Newfoundland. Acarologia 16, 621–635.
Feil, E.J., Enright, M.C., 2004. Analyses of clonality and the evolution of bacterial pathogens. Curr. Opin. Microbiol. 7, 308–313. https://doi.org/10.1016/j.mib.2004.04.002.
Francisco, A.P., Bugalho, M., Ramirez, M., Carriço, J.A., 2009. Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. BMC Bioinform. 10, 152. https://doi.org/10.1186/1471-2105-10-152.
Francisco, A.P., Vaz, C., Monteiro, P.T., Melo-Cristino, J., Ramirez, M., Carrico, J.A., 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. BMC Bioinform. 13, 87. https://doi.org/10.1186/1471-2105-13-87.
Frederiksen, M., Descamps, S., Erikstad, K.E., Gaston, A.J., Gilchrist, H.G., Grémillet, D., Johansen, K.L., Kolbeinsson, Y., Linnebjerg, J.F., Mallory, M.L., McFarlane Tranquilla, L.A., Merkel, F.R., Montevecchi, W.A., Mosbech, A., Reiertsen, T.K., Robertson, G.J., Steen, H., Strøm, H., Thórarinsson, T.L., 2016. Migration and wintering of a declining seabird, the thick-billed murre *Uria lomvia*, on an ocean basin scale: conservation implications. Biol. Conserv. 200, 26–35. https://doi.org/10.1016/j.biocon.2016.05.011.
Frederiksen, M., Moe, B., Daunt, F., Phillips, R.A., Barrett, R.T., Bogdanova, M.I., Boulinier, T., Chardine, J.W., Chastel, O., Chivers, L.S., Christensen-Dalsgaard, S., Clément-Chastel, C., Colhoun, K., Freeman, R., Gaston, A.J., González-Solís, J., Goutte, A., Grémillet, D., Guilford, T., Jensen, G.H., Krasnov, Y., Lorentsen, S.-H., Mallory, M.L., Newell, M., Olsen, B., Shaw, D., Steen, H., Strøm, H., Systad, G.H., Thórarinsson, T.L., Anker-Nilssen, T., 2012. Multicolony tracking reveals the winter distribution of a pelagic seabird on an ocean basin scale. Divers. Distrib. 18, 530–542. https://doi.org/10.1111/j.1472-4642.2011.00864.x.
Fukunaga, M., Takahashi, Y., Tsuruta, Y., Matsushita, O., Ralph, D., McClelland, M., Nakao, M., 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int. J. Syst. Bacteriol. 45, 804–810. https://doi.org/10.1099/00207713-45-4-804.
Gern, L., Humair, P.F., 2002. Ecology of *Borrelia burgdorferi* sensu lato in Europe. In: Gray, J.S., Kahl, O., Lane, R.S., Stanek, G. (Eds.), Lyme Borreliosis: Biology, Epidemiology and Control. CABI Publishing, Wallingford, Oxon, UK, pp. 149–348.
Gomez-Diaz, E., Boulinier, T., Sertour, N., Cornet, M., Ferquel, E., McCoy, K.D., 2011. Genetic structure of marine *Borrelia garinii* and population admixture with the terrestrial cycle of Lyme borreliosis. Environ. Microbiol. 13, 2453–2467. https://doi.org/10.1111/j.1462-2920.2011.02515.x.
Gomez-Diaz, E., Doherty Jr., P.F., Duneau, D., McCoy, K.D., 2010. Cryptic vector divergence masks vector-specific patterns of infection: an example from the marine cycle of Lyme borreliosis. Evol. Appl. 3, 391–401. https://doi.org/10.1111/j.1752-4571.2010.00127.x.
Goudet, J., 2005. Hierfstat, a package for r to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.
Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. https://doi.org/10.1093/sysbio/syq010.
Gylfe, A., Bergström, S., Lundström, J., Olsen, B., 2000. Reactivation of *Borrelia* infection in birds. Nature 403, 724–725. https://doi.org/10.1038/35001663.
Gylfe Olsen, B., Strasevicius, D., Marti Ras, N., Weihe, P., Noppa, L., Ostberg, Y., Baranton, G., Bergström, S., 1999. Isolation of Lyme disease *Borrelia* from puffins (*Fratercula arctica*) and seabird ticks (*Ixodes uriae*) on the Faeroe Islands. J. Clin. Microbiol. 37, 890–896.
Harris, M.P., 1980. Breeding performance of puffins *Fratercula arctica* in relation to nest density, laying date and year. Ibis 120, 193–209.
Harris, M.P., Birkhead, T.R., 1985. Breeding ecology of the Atlantic Alcidae. In: Nettleship, D.N., Birkhead, T.R. (Eds.), The Atlantic Alcidae: Evolution, Distribution and Biology of the Auks Inhabiting the Atlantic Ocean and Adjacent Water Areas. Academic Press, London, UK, pp. 156–204.
Hatch, S.A., Robertson, G.J., Baird, P.H., 2009. Black-legged Kittiwake (*Rissa tridactyla*). In: Rodewald, P.G. (Ed.), The Birds of North America. Cornell Lab of Ornithology, Ithaca, NY. https://doi.org/10.2173/bna.92.
Hellgren, O., Andersson, M., RÅBerg, L., 2011. The genetic structure of *Borrelia afzelii* varies with geographic but not ecological sampling scale. J. Evol. Biol. 24, 159–167. https://doi.org/10.1111/j.1420-9101.2010.02148.x.
James, M.C., Gilbert, L., Bowman, A.S., Forbes, K.J., 2014. The heterogeneity, distribution, and environmental associations of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. Front. Public Health 2, 129. https://doi.org/10.3389/fpubh.2014.00129.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinform. 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.
Kempf, F., Boulinier, T., De Meeûs, T., Arnathau, C., McCoy, K.D., 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. Mol. Ecol. 18, 4450–4462. https://doi.org/10.1111/j.1365-294X.2009.04356.x.
Kurtenbach, K., De Michelis, S., Etti, S., Schäfer, S.M., Sewell, H.S., Brade, V., Kraiczy, P., 2002. Host association of *Borrelia burgdorferi* sensu lato - the key role of host complement. Trends Microbiol. 10, 74–79. https://doi.org/10.1016/S0966-842X(01)02298-3.
Kurtenbach, K., Hanincová, K., Tsao, J.I., Margos, G., Fish, D., Ogden, N.H., 2006. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat. Rev. Microbiol. 4, 660–669. https://doi.org/10.1038/nrmicro1475.
Lavers, J., Hipfner, J.M., Chapdelaine, G., 2009. Razorbill (*Alca torda*). In: Rodewald, P.G. (Ed.), The Birds of North America. Cornell Lab of Ornithology, Ithaca, NY. https://doi.org/10.2173/bna.635.
Lowther, P.E., Diamond, A.W., Kress, S.W., Robertson, G.J., Russell, K., 2002. Atlantic Puffin (*Fratercula arctica*). In: Rodewald, P.G. (Ed.), The Birds of North America. Cornell Lab of Ornithology, Ithaca, NY. https://doi.org/10.2173/bna.709.
Margos, G., Gatewood, A.G., Aanensen, D.M., Hanincová, K., Terekhova, D., Vollmer, S.A., Cornet, M., Piesman, J., Donaghy, M., Bormane, A., Hurn, M.A., Feil, E.J., Fish, D., Casjens, S., Wormser, G.P., Schwartz, I., Kurtenbach, K., 2008. MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. U. S. A. 105, 8730–8735. https://doi.org/10.1073/pnas.0800323105.
Margos, G., Lane, R.S., Fedorova, N., Koloczek, J., Piesman, J., Hojgaard, A., Sing, A., Fingerle, V., 2016. *Borrelia bissettiae* sp. nov. and *Borrelia californiensis* sp. nov. prevail in diverse enzootic transmission cycles. Int. J. Syst. Evol. Microbiol. 66, 1447–1452. https://doi.org/10.1099/ijsem.0.000897.
Margos, G., Piesman, J., Lane, R.S., Ogden, N.H., Sing, A., Straubinger, R.K., Fingerle, V., 2014. *Borrelia kurtenbachii* sp. nov., a widely distributed member of the *Borrelia burgdorferi* sensu lato species complex in North America. Int. J. Syst. Evol. Microbiol. 64, 128–130. https://doi.org/10.1099/ijs.0.054593-0.
Margos, G., Tsao, J.I., Castillo-Ramírez, S., Girard, Y.A., Hamer, S.A., Hoen, A.G., Lane, R.S., Raper, S.L., Ogden, N.H., 2012. Two boundaries separate *Borrelia burgdorferi* populations in North America. Appl. Environ. Microbiol. 78, 6059–6067. https://doi.org/10.1128/AEM.00231-12.
Margos, G., Vollmer, S.A., Cornet, M., Garnier, M., Fingerle, V., Wilske, B., Bormane, A., Vitorino, L., Collares-Pereira, M., Drancourt, M., Kurtenbach, K., 2009. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl. Environ. Microbiol. 75, 5410–5416. https://doi.org/10.1128/AEM.00116-09.
Margos, G., Wilske, B., Sing, A., Hizo-Teufel, C., Cao, W.C., Chu, C., Scholz, H., Straubinger, R.K., Fingerle, V., 2013. *Borrelia bavariensis*sp. nov. is widely distributed in Europe and Asia. Int. J. Syst. Evol. Microbiol. 63, 4284–4288. https://doi.org/10.1099/ijs.0.052001-0.
McCoy, K.D., 2008. The population genetic structure of vectors and our understanding of disease epidemiology. Parasite 15, 444–448. https://doi.org/10.1051/parasite/2008153444.
McCoy, K.D., Boulinier, T., Chardine, J.W., Danchin, E., Michalakis, Y., 1999. Dispersal and distribution of the tick *ixodes uriae* within and among seabird host populations: the need for a population genetic approach. J. Parasit. 85, 196–202.
McCoy, K.D., Boulinier, T., Schjørring, S., 2002. Local adaptation of the ectoparasite *Ixodes uriae* to its seabird host. Evol. Ecol. Res. 4, 441–456.
Mechai, S., Feil, E.J., Gariepy, T.D., Gregory, T.R., Lindsay, L.R., Millien, V., Ogden, N.H., 2013. Investigation of the population structure of the tick vector of Lyme disease *Ixodes scapularis* (Acari: Ixodidae) in Canada using mitochondrial cytochrome C oxidase subunit I gene sequences. J. Med. Entomol. 50, 560–570. https://doi.org/10.1603/ME12178.
Mechai, S., Margos, G., Feil, E.J., Barairo, N., Lindsay, L.R., Michel, P., Ogden, N.H., 2016. Evidence for host-genotype associations of *Borrelia burgdorferi* sensu stricto. PLoS One 11, e149345. https://doi.org/10.1371/journal.pone.0149345.
Mechai, S., Margos, G., Feil, E.J., Lindsay, L.R., Ogden, N.H., 2015. Complex population structure of *Borrelia burgdorferi* in southeastern and south central Canada as revealed by phylogeographic analysis. Appl. Environ. Microbiol. 81, 1309–1318. https://doi.org/10.1128/aem.03730-14.
Munro, H.J., Ogden, N.H., Lindsay, L.R., Robertson, G.J., Whitney, H., Lang, A.S., 2017. Evidence for *Borrelia bavariensis* infections of *Ixodes uriae* within seabird colonies of the North Atlantic Ocean. Appl. Environ. Microbiol. 83, e01087–17. https://doi.org/10.1128/aem.01087-17.
Muzaffar, S.B., Smith, R.P., Jones, I.L., Lavers, J., Lacombe, E.H., Cahill, B.K., Lubelczyk, C.B., Rand, P.W., 2012. The trans-Atlantic movement of the spirochete *Borrelia garinii*: the role of ticks and their seabird hosts. J. Avian Biol. 42, 23–30. https://doi.org/10.3201/eid1212.060448.
Ogden, N.H., Margos, G., Aanensen, D.M., Drebot, M.A., Feil, E.J., Hanincova, K., Schwartz, I., Tyler, S., Lindsay, L.R., 2011. Investigation of genotypes of *Borrelia burgdorferi* in *Ixodes scapularis* ticks collected during surveillance in Canada. Appl. Environ. Microbiol. 77, 3244–3254. https://doi.org/10.1128/AEM.02636-10.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. Vegan: Community Ecology Package.
Olsen, B., Duffy, D.C., Jaenson, T.G., Gylfe, A., Bonnedahl, J., Bergström, S., 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. J. Clin. Microbiol. 33, 3270–3274.
Olsen, B., Jaenson, T.G., Noppa, L., Bunikis, J., Bergström, S., 1993. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. Nature 362, 340–342. https://doi.org/10.1038/362340a0.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25,

1253–1256. https://doi.org/10.1093/molbev/msn083.
Postic, D., Garnier, M., Baranton, G., 2007. Multilocus sequence analysis of atypical *Borrelia burgdorferi* sensu lato isolates – description of *Borrelia californiensis* sp. nov., and genomospecies 1 and 2. Int. J. Med. Microbiol. 297, 263–271. https://doi.org/10.1016/j.ijmm.2007.01.006.
Qiu, W.G., Martin, C.L., 2014. Evolutionary genomics of *Borrelia burgdorferi* sensu lato: findings, hypotheses, and the rise of hybrids. Infect. Genet. Evol. 27, 576–593. https://doi.org/10.1016/j.meegid.2014.03.025.
Qiu, W.-G.G., Dykhuizen, D.E., Acosta, M.S., Luft, B.J., 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the Northeastern United States. Genet 160, 833–849.
R Core Team, 2017. R: A Language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Radolf, J.D., Caimano, M.J., Stevenson, B., Hu, L.T., 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat. Rev. Microbiol. 10, 87–99. https://doi.org/10.1038/nrmicro2714.
Richter, D., Schlee, D.B., Allgöwer, R., Matuschka, F.R., 2004. Relationships of a novel Lyme disease spirochete, *Borrelia spielmani* sp. nov., with its hosts in Central Europe. Appl. Environ. Microbiol. 70, 6414–6419. https://doi.org/10.1128/AEM.70.11.6414-6419.2004.
Smith, R.P., Muzaffar, S.B., Lavers, J., Lacombe, E.H., Cahill, B.K., Lubelczyk, C.B., Kinsler, A., Mathers, A.J., Rand, P.W., 2006. *Borrelia garinii* in seabird ticks (*Ixodes uriae*), Atlantic Coast, North America. Emerg. Infect. Dis. 12, 1909–1912. https://doi.org/10.3201/eid1212.060448.
Stanek, G., Strle, F., 2003. Lyme borreliosis. Lancet 362, 1639–1647. https://doi.org/10.1016/s0140-6736(03)14798-8.
Takezaki, N., Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genet 144, 389–399.
Tuck, L.M., 1960. The Murres. Canadian Wildlife Service, Ottawa, Canada.
Vollmer, S.A., Bormane, A., Dinnis, R.E., Seelig, F., Dobson, A.D., Aanensen, D.M., James, M.C., Donaghy, M., Randolph, S.E., Feil, E.J., Kurtenbach, K., Margos, G., 2011. Host migration impacts on the phylogeography of Lyme borreliosis spirochaete species in Europe. Environ. Microbiol. 13, 184–192. https://doi.org/10.1111/j.1462-2920.2010.02319.x.
Vollmer, S.A., Feil, E.J., Raper, S.L., Kurtenbach, K., Chu, C.-Y., Cao, W.-C., Margos, G., 2013. Spatial spread and demographic expansion of Lyme borreliosis spirochaetes in Eurasia. Infect. Genet. Evol. 14, 47–155. https://doi.org/10.1016/j.meegid.2012.11.014.
Wang, G., Liveris, D., Mukherjee, P., Jungnick, S., Margos, G., Schwartz, I., 2014. Molecular typing of *Borrelia burgdorferi*. Curr. Protoc. Microbiol. 34, 12C.5.1–12C.5.31. https://doi.org/10.1002/9780471729259.mc12c05s34.
Woolhouse, M.E., Webster, J.P., Domingo, E., Charlesworth, B., Levin, B.R., 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nat. Genet. 32, 569–577. https://doi.org/10.1038/ng1202-569.