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Prevalence and diversity of Lyme borreliosis bacteria in marine birds☆

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ABSTRACT

A potential role of seabirds in spreading Lyme disease (LB) spirochetes over large spatial scales was suggested more than 10 years ago when *Borrelia garinii* was observed in marine birds of both hemispheres. Since then, there have been few studies examining the diversity of *Borrelia* spp. circulating in seabirds, or the potential interaction between terrestrial and marine disease cycles. To explore these aspects, we tested 402 *Ixodes uriae* ticks collected from five colonial seabird species by amplification of the *flaB* gene. Both the average prevalence (26.0% ± 3.9) and diversity of LB spirochetes was high. Phylogenetic analyses grouped marine isolates in two main clades: one associated with *B. garinii* and another with *B. lusitaniae*, a genospecies typically associated with lizards. One sequence also clustered most closely with *B. burgdorferi* sensu stricto. Prevalence in ticks varied both among seabird species within colonies and among colonies. However, there was no clear association between different *Borrelia* isolates and a given seabird host species. Our findings indicate that LB spirochetes circulating in the marine system are more diverse than previously described and support the hypothesis that seabirds may be an important component in the global epidemiology and evolution of Lyme disease. Future work should help determine the extent to which isolates are shared between marine and terrestrial systems.

1. Introduction

The important role that birds play in the transmission of medically significant zoonoses is becoming increasingly apparent (e.g., Comstedt et al., 2006; Poupon et al., 2006). Migratory birds (passerines, waterfowl) are known to carry numerous pathogenic agents of human and livestock disease (West Nile Virus, Influenza, Anaplasma, etc.), including several of the bacterial agents responsible for Lyme disease. Lyme disease is the most common vector-borne zoonosis in temperate regions of the Northern hemisphere and has significant medical and economic impacts (e.g., Zhang et al., 2006). The causative agent of this disease belongs to the bacterial complex *Borrelia burgdorferi* sensu lato, also referred to as Lyme borreliosis (LB) spirochetes. This complex currently contains at least 13 described genomic groups or genospecies: *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. japonica*, '*B. andersonii*', *B. tanukii*, *B. turdi*, *B. valaisiana*, *B. lusitaniae*, '*B. bissettii*', *B. sinica*, '*B. californiensis*' and *B. spielmanii* (Richter et al., 2006; Postic et al., 2007). Among these genospecies, four (*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* and *B. spielmanii*) are associated with human Lyme disease (Fingerle et al., 2006) and evidence now suggests that two others may also be pathogenic under certain circumstances: *B. valaisiana* (Godfroid et al., 2003) and *B. lusitaniae* (Collares-Pereira et al., 2004). The principal vectors of this disease are considered to be four tick species of the genus *Ixodes*: *I. scapularis* and *I. pacificus* in North America, *I. ricinus* in Europe and *I. persulcatus* in Asia. In these species, infection prevalence in questing ticks can be high (e.g., up to 33% of nymphs in Alsace, France; Ferquel et al., 2006) and contact with humans frequent (Eisen and Lane, 2002). However, natural infections by *B. burgdorferi* s.l. have been recorded in 25 other ixodid ticks and

☆ Note: Nucleotide sequence data reported in this paper will be available in the GenBank database under the accession numbers EF154357–EF154390; EU567327–EU567329.

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vectorial competence has been confirmed experimentally for at least 8 of these species (see Eisen and Lane, 2002 for a review). Although not frequently considered to be major bridging vectors of Lyme disease spirochetes to humans, these species may still play an important role in the maintenance and spread of the disease agent at different spatial scales. In order to better understand the global epidemiology of Lyme disease, determining the relative importance of these alternative tick hosts in the enzootic cycle requires more explicit consideration (Kurtenbach et al., 2006).

More than 10 years ago, *B. burgdorferi* s.l. was detected for the first time in the seabird tick, *Ixodes uriae*, and a potential role for seabirds in Lyme disease epidemiology was suggested (Olsen et al., 1993). More in-depth studies revealed that this bacterium was present in ticks from seabird colonies in both the northern and southern hemispheres and identified the bacterium as *Borrelia garinii* (Olsen et al., 1995). A study in the Faeroe Islands then demonstrated that seabirds were indeed competent reservoirs of this disease agent and that humans could be infected when in contact with the seabird tick (Gylfe et al., 1999). For now, only one genospecies, *B. garinii*, has been found circulating in the seabird-tick system.

Of the different alternative vector species that may be involved in Lyme disease epidemiology, *I. uriae* may play a particularly important role in the maintenance and spread of the disease. *I. uriae* has been recorded on more than 50 different colonial seabird species in the polar areas of both hemispheres (McCoy et al., 2005). Although previously considered as a seabird generalist, this tick has since been shown to recurrently form distinct races associated with one or few closely related seabird host species; therefore local populations structured by host species seems to be the general rule (McCoy et al., 2001, 2005). This type of isolation may lead to different infection dynamics in different seabird species and to the maintenance of several semi-independent disease cycles. Despite the fact that *I. uriae* is widespread, highly abundant, and that seabirds can migrate over extremely large spatial scales, the role of these hosts and their ticks in the epidemiology of Lyme disease has received relatively little attention since the first detection of the bacterium (Olsen et al., 1993, 1995).

To improve our understanding of the overall importance of seabirds as LB reservoirs and to determine their potential role in the evolution and global epidemiology of this disease agent, we investigated the prevalence and genetic diversity of *B. burgdorferi* s.l. circulating in seabird colonies by sampling ticks from five different seabird species. We analyze and discuss both spatial and host-associated factors that might explain the distribution of LB spirochetes in this system along with the epidemiological implications of our results.

2. Materials and methods

2.1. Tick sampling

I. uriae was collected in four large seabird colonies in the North Atlantic, three in Iceland sampled in 2003 and one in northern Norway, sampled in 1998 (Fig. 1). In these colonies, ticks were collected directly from the seabirds in the breeding areas. Five host species were sampled: black-legged kittiwake *Rissa tridactyla*, Atlantic puffin *Fratercula arctica*, common guillemot *Uria aalge*, razorbill *Alca torda* and northern fulmar *Fulmarus glacialis*. Previous studies, including ticks from the Norwegian colony, revealed that *I. uriae* has formed seabird specific host races (McCoy et al., 2001, 2005). For this reason, ticks collected from different host species within the same colony were treated as being from independent populations. On Hornøya (70°22′N, 31°10′E), ticks were collected from three seabird species breeding sympatrically (kittiwake, puffin and guillemot). On the Icelandic islands of Skrudur (64°54′N, 13°38′W) and Grimsey (66°33′N, 18°00′W), these different seabirds were also sympatric and, on Grimsey, ticks were also collected from razorbills. In the Breidafjordur (Fig. 1), the configuration of the seabird colonies are more complex and widespread; ticks from guillemots and razorbills were sampled along the cliffs of Latrabjarg (65°29′N, 24°32′W), from kittiwakes and puffins on the small islet of Hrolfsklettur (65°23′N, 22°54′W) and from fulmars on another islet, Hafnarey (65°23′N, 22°55′W). In each colony, we attempted to sample at least 30 adult or nymphal ticks from as many individual birds of each seabird species (Table 1). After collection, ticks were stored in 70–90% ethanol until DNA extractions.

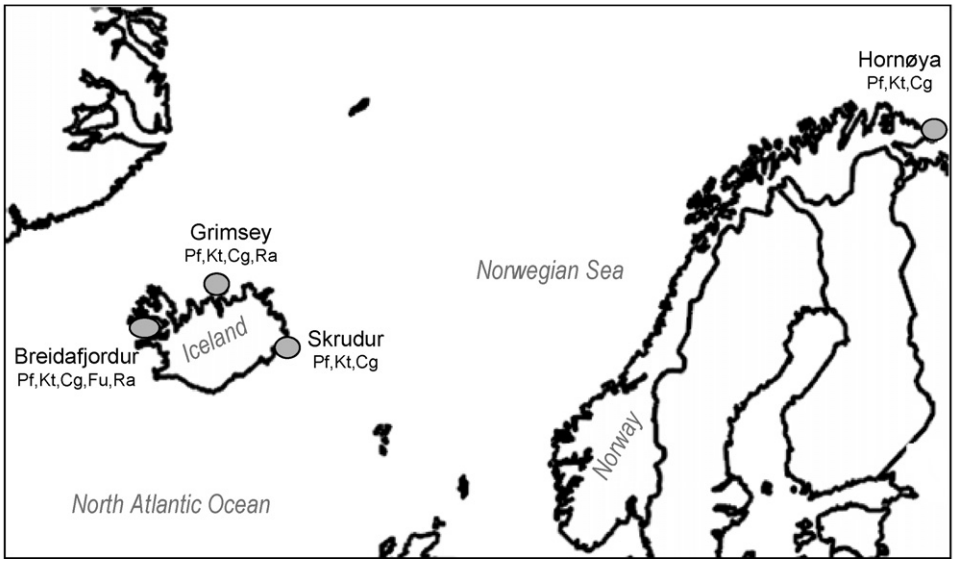

Fig. 1. Location of seabird colonies sampled in northern Europe (Iceland and Norway). In each location, ticks were collected from several local seabird species. The sampled species are indicated by abbreviations (Pf: Atlantic puffin, Kt: black-legged kittiwake, Cg: common guillemot, Fu: northern fulmar, Ra: razorbill). Note that all host species were sympatric except in the Breidafjordur; in this area, three different sub-colonies were sampled with Pf and Kt in one breeding site, CG and Ra in another, and Fu in the third.

Table 1
Prevalence and distribution of *Borrelia burgdorferi* s.l. isolates

Colony	Host (no. birds sampled)	No. of ticks (no. adults, no. nymphs)	Prevalence of *Borrelia*		Strain distribution[a]		
			No. positive ticks (no. hosts)[b]	Colony avg.	Grp 1	Grp 2	Co-inf
Hornøya (Norway)	Kittiwake (21)	29 (26A, 3N)	2 (2)			1	
	Puffin (27)	30 (9A, 25N)	7 (7)			5	
	Guillemot (29)	30 (28A, 2N)	7 (7)			4	
			Total: 16	18.0%			
Skrudur (Iceland)	Kittiwake (17)	28 (25A, 3N)	5 (5)		2	2	1
	Puffin (19)	31 (26A, 5N)	7 (4)			7	
	Guillemot (27)	32 (32A)	6 (6)		2	4	
			Total: 18	19.8%			
Grimsey (Iceland)	Kittiwake (24)	30 (16A, 14N)	10 (10)		2	5	3
	Puffin (28)	39 (29A, 10N)	13 (11)		2	11	
	Guillemot (23)	30 (25A, 5N)	10 (10)		3	5	2
	Razorbill (12)	17 (1A, 16N)	0				
			Total: 33	28.4%			
Breidafjordur (Iceland)	Kittiwake (11)	20 (2A, 18N)	9 (8)		2	6	1
	Puffin (25)	30 (14A, 16N)	19 (16)			13	2
	Guillemot (7)	12 (12A)	3 (3)			1	1
	Razorbill (9)	14 (12A, 2N)	2 (2)			2	
	Fulmar (16)	30 (11A, 19N)	9 (9)			6	1
			Total: 42	39.6%			

The strain groups refer to clades identified on the phylogenetic tree (see Fig. 2) and 'co-inf' indicates the number of ticks infected with more than one *Borrelia* sp. strain. See Fig. 1 for colony locations.

[a] Clear sequences were not obtained for 12 positive individuals. One isolate of *B. burgdorferi* sensu stricto was found for a guillemot tick on Hornøya.

[b] No. hosts refers to the number of sampled hosts with at least one infected tick.

2.2. DNA extractions, nested PCR procedures and sequencing

DNA extractions were performed using a DNeasy Tissue Kit (Qiagen, Valencia, CA). Conserved ticks were cut in half so as to include part of the gut and the salivary glands. To eliminate any traces of ethanol, the piece of tick was placed in an open 1.5 ml tube overnight under a fume hood. A steel bead was then added to the tube and ticks were frozen using liquid nitrogen and ground with a mixer mill 301 (Retsch, Germany). Extractions were then performed following the kit procedures. DNA was eluted in 100 μl of AE buffer and was subsequently diluted on the basis of a spectrophotometric analysis to standardize the amount used in PCRs.

To determine if a tick was infected by LB spirochetes, we used a nested PCR procedure for the amplification of the *flaB* gene using primers designed to amplify all strains of *B. burgdorferi* sensu lato (Johnson et al., 1992). The *flaB* gene encodes a 41 kDa flagellin protein and is located on the linear chromosome. This gene was chosen to maximize the probability of detecting infection by LB spirochetes. During the first PCR, a 611-pb portion of the gene was amplified with primers outer1 and outer2 (Johnson et al., 1992). A small amount (0.5 μl) of the amplicon from the first PCR was then used as the template in the second PCR. The second PCR used primers inner1 and inner2 and enabled the amplification of a 390-pb sequence of the polymorphic region of this gene (Gassman et al., 1989). Each 25 μl reaction mixture was composed of 2.5 μl 10× buffer (Tris–HCl, pH 9.0, KCl, Triton® X-100), 2 μl $MgCl_2$ (25 mM), 2 μl dNTP (2.5 mM), 0.5 μl forward primer (20 μM), 0.5 μl reverse primer (20 μM), 1.25 U Taq polymerase (Promega), 20–50 ng DNA and sterile, distilled water. The PCR conditions consisted of an initial denaturation step at 95 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The second PCR followed the same program, except that the annealing temperature was raised to 55 °C. All PCRs were run with both positive (DNA from cultured *B. garinii*-200047 or *B. lusitaniae*-POtib2) and negative (distilled water) controls. The final 390-bp PCR products were visualized on 2% agarose gels using ethidium bromide. All positive amplifications were re-amplified and sent for direct sequencing (Genome Express, Meylan France).

2.3. Prevalence analyses

Differences in prevalence among tick races within each colony were tested using Fisher's exact tests. The results of the independent tests were then summarized using Fisher's combination test procedure (Raymond and Rousset, 1995). We tested for overall differences in prevalence among colonies using a non-parametric multiple means test (Kruskal–Wallis test) and considered only those tick races found in all four colonies (kittiwake, puffin, guillemot).

2.4. Phylogenetic analyses

In order to determine the relationship of the detected strains of LB spirochetes to known genospecies, we carried out a phylogenetic analysis. The freeware Seaview (Galtier et al., 1996) was used to align the *flaB* gene sequences from our samples along with 24 sequences obtained from Genbank (http://www.ncbi.nlm.nih.gov) (see Table 2 for details and accession numbers). If available, we selected two divergent reference sequences from each of 13 currently described genospecies of the *B. burgdorferi* s.l. complex. A *Borrelia hermsii* sequence, a relapsing fever *Borrelia*, was used as the outgroup (e.g., Fukunaga et al., 1996). We used MODELTEST 3.6 (Posada and Crandall, 1998) to search for the best-fit model of nucleotide substitution for our sequence data. Given the likelihood scores of the hierarchical likelihood ratio test, we selected the model HKY + G using the empirically determined base frequencies, the discrete gamma distribution and the substitution model for among site variation. This model was applied in a maximum likelihood phylogenetic analysis using PAUP*4.0b10 (Swofford, 2002) and the resulting tree was drawn using TREEDYN (Chevenet et al., 2006). Reliability of the ML trees was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications.

To examine if different *Borrelia* isolates were associated with a particular host species, we constructed a split network to represent

Table 2
Reference sequences used in the phylogenetic analysis (see Fig. 2)

Species	Isolate	Genbank accession no.
Borrelia afzelii	Iper3	AY342020
	ACA1	AB035613
Borrelia andersonii	21038	D83763
	19857	D83762
Borrelia bissettii	CA128	DQ393343
Borrelia burgdorferi sensu stricto	B31	X15661
	GeHo	X15660
Borrelia californiensis	CA443	DQ393348
	CA404	DQ393346
Borrelia garinii	Ip90	L42885
	20047	D82846
Borrelia japonica	NT112	D82853
	HO14	D82852
Borrelia lusitaniae	47ZLIM	DQ788619
	D23-04	DQ016623
Borrelia sinica	CMN1a	AB022137
	CMN3	AB022138
Borrelia spielmanii	PC-Eq2/1	AY450560
Borrelia tanukii	Hk501	D82847
	OR1eR	D85070
Borrelia turdi	Kt501	D82851
Borrelia valaisiana	OS66/01	AB091715
	CMN1b	AB022134
Borrelia hermsii (outgroup)	YOR	AY597806

[a]Unnamed in the Genbank file, but now recognized as *B. bissettii* (Postic et al., 1998).

the genealogical relationships among *Borrelia* isolates on which we mapped either seabird host species or geographic location. The split network was constructed using the split decomposition algorithm implemented in SplitsTree v4.6 and is designed to represent phylogenetic incompatibilities within the data set (Huson and Bryant, 2006).

3. Results

3.1. Prevalence

Among the 402 ticks tested, we found 109 positive for *B. burgdorferi* sensu lato. The bacterium was present in ticks of all four colonies and infected ticks were recorded from all five seabird species (Table 1). The mean prevalence across colonies was 26.0% (±3.9), but within each colony, the difference among host races was variable. There was no difference in prevalence among host races sampled on Skrudur (Fisher's exact test, $p = 0.895$). On Hornøya a difference was suggested, but was not statistically significant (Hornøya $p = 0.18$). However, prevalence did vary significantly among tick races on Grimsey ($p = 0.019$) and in the Breidafjordur ($p = 0.011$). When results of the different tests were combined, overall prevalence was significantly different among tick races within colonies (Fisher's combined test, $p = 0.0081$). When considering only those host races sampled in all colonies, overall prevalence also varied significantly among colonies (Kruskal–Wallis test, $p = 0.001$), with the highest prevalence of infected ticks in Breidafjordur and the lowest on Hornøya (Table 1). The presence of several ticks sampled from the same host individual (Table 1) could bias prevalence if the sampled host was the source of infection. However, this was not a significant factor in the present study as most positive ticks came from different host individuals or carried different *Borrelia* isolates (Table 1). If we reduce the dataset to include only a single tick per host individual, selected randomly with respect to infection status, we find the same results (e.g., average prevalence = 28.0% ± 4.6).

3.2. Phylogenetic analyses

Ambiguous sequences were found for 11 samples due to infection by more than one *Borrelia* strain (Table 1); we were not able to resolve the individual sequences and they are therefore not included in the subsequent analyses. There were also 12 amplifications that were not of sufficient quality to unambiguously resolve the sequence. These sequences were also excluded from the phylogenetic analysis. There were 31 different sequences found among the remaining 86 positive samples (Genbank accession numbers EF154357–EF154390; EU567327–EU567329). After alignment, a 311-pb region was used for the phylogenetic analysis. There were 63 variable sites of which 21 were informative. Phylogenetic tree construction included all 31 sequences from our samples along with the 24 reference sequences (Table 2). A given sequence was only included once in tree construction to avoid giving disproportionate weight to certain mutational events. The sequences from the present study clustered into two well-supported clades (Fig. 2: groups 1 and 2). The majority of sequenced strains grouped with reference sequences of *B. garinii* (Fig. 2); a small internal clade that included nine isolates from this study along with the two reference sequences was apparent in the tree, but did not receive strong bootstrap support (67%). Thirteen sequences grouped with *Borrelia lusitaniae* (group 1) and one sequence, isolated from a guillemot tick on Hornøya grouped most closely with *B. burgdorferi* sensu stricto. In no case were sequences identical to the positive controls used in the PCRs. As the robustness of the internal nodes of the tree was low, we were not able to resolve the evolutionary relationships among clades. The split network mirrored the structure of the phylogenetic tree with few incompatibilities. However, no obvious association between *Borrelia* strains and host type was visible (Fig. 3). This was also the case when geographic location was mapped onto the network (results not shown).

4. Discussion

The critical role of birds as Lyme disease reservoirs is becoming increasingly obvious (e.g., Hanincova et al., 2003; Comstedt et al., 2006; Kipp et al., 2006; Poupon et al., 2006). Interestingly, the strains associated with birds often show greater polymorphism than those limited to mammals only (Ras et al., 1997; Wang et al., 1999). This suggests that birds may not only maintain and disseminate this bacterium, but that they may also be an important source of new variants. Here, we investigated the poorly known enzootic Lyme disease cycle that involves colonial seabirds and their associated tick, *I. uriae*. We determined the prevalence and distribution of LB spirochetes across a range of colonies and seabird species in order to examine factors that might affect the ecology and evolution of this bacterial complex and to consider the potential importance of this cycle for the global epidemiology of Lyme disease.

We found that the overall prevalence of LB spirochetes was high in the marine system (~26%) and varied significantly among ticks sampled from different seabird species within the same colony. However, there were few general tendencies in terms of which host species might be the main reservoir maintaining this pathogen; only ticks from razorbills had relatively low infection prevalence, but few samples were available for this seabird species. Previous studies have found LB spirochetes in association with guillemots, puffins and razorbills. In addition to detection in the tick vector, these bacteria have also been directly isolated from skin biopsies and blood samples from these birds (Olsen et al., 1993; Gylfe et al., 1999). Immunological studies have also shown that the prevalence of *Borrelia*-specific antibodies is high in general and varies significantly among different sympatric seabird species and among colonies (Gasparini et al., 2001; Staszewski, 2007). However, this is the first time that *B. burgdorferi* s.l. isolates have been found in association with black-legged kittiwakes and northern fulmars, two species with particularly broad

Fig. 2. Phylogenetic tree of the ***Borrelia flaB*** sequences using maximum likelihood. Reference sequences were obtained from Genbank and are listed in Table 2. Bootstrap analysis (1000 repeats) was performed to evaluate the robustness of branches and only values greater than 75% are indicated on the tree. The sequences from this study are referred to by a "T" followed by a simple 3 or 4-digit number. Several copies of a given sequence were sometimes found; the total number is indicated in brackets. Overall, samples grouped into two well-supported clades: group 1 clustered with ***B. lusitaniae***, whereas group 2 isolates were most closely related to ***B. garinii***. One sequence grouped with ***B. burgdorferi*** sensu stricto.

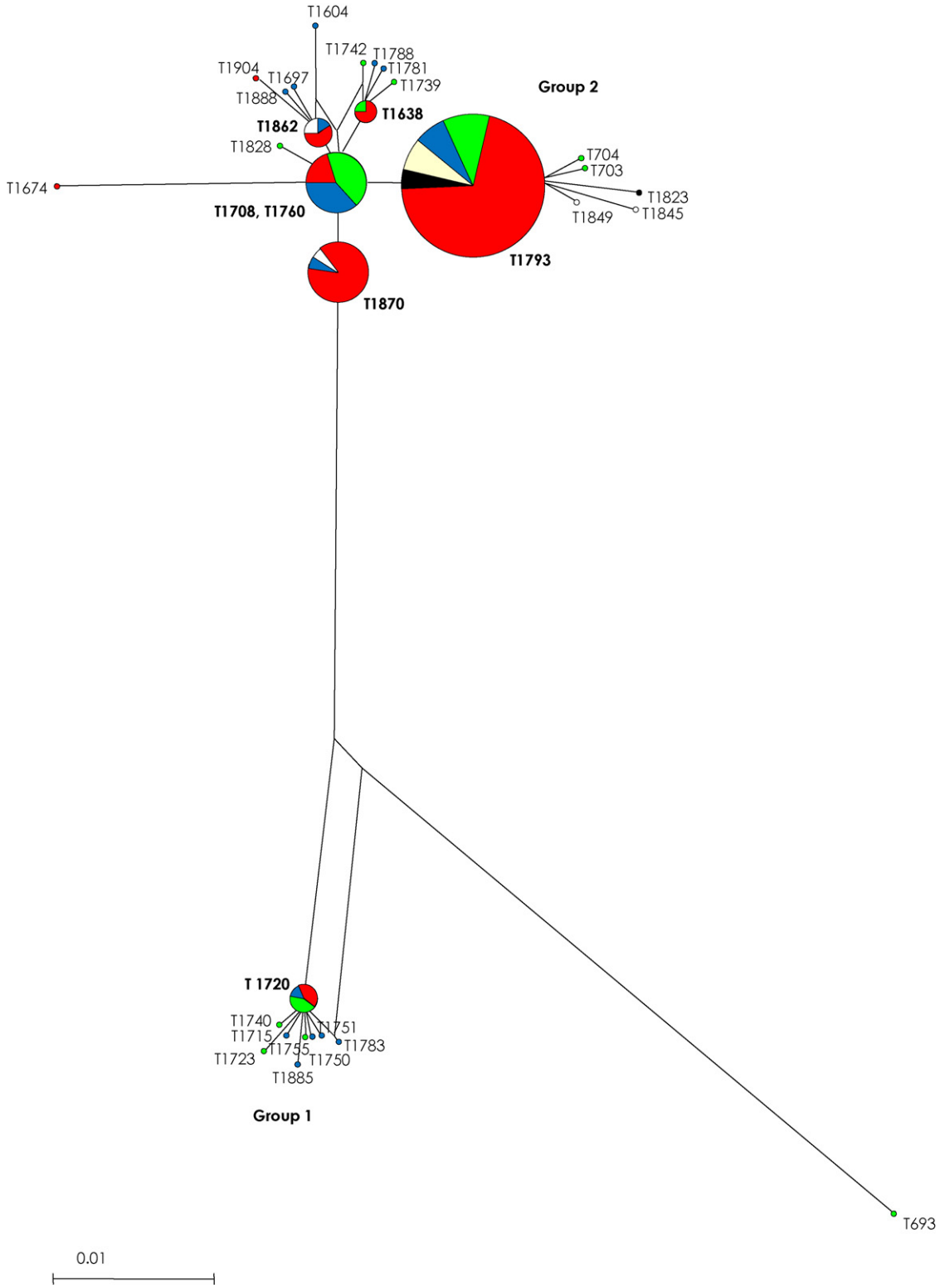

Fig. 3. Split network showing the genetic relationships among ***Borrelia flab*** sequences. Circle size is proportional to the number of tick individuals sharing identical strains and main haplotypes are in bold. Numerical labels and groups are the same as those indicated on Fig. 2. The host species associated with each infected tick are indicated in colour ((●) puffin, (●) guillemot, (●) kittiwake, (●) razorbill and (○) fulmar).

distributions. These findings therefore confirm that LB spirochetes are widespread and abundant in colonial seabirds. Although finding spirochetes in ticks does not demonstrate that all of the host species examined here are competent reservoirs, this is likely for several reasons. First, as *I. uriae* tends to form host-specific races across its range (McCoy et al., 2001, 2005), different sympatric seabird species will not likely share the same tick population. In addition, this tick is nidicolous and only feeds on birds at the nest site. As these birds are highly faithful to their nest site over time (e.g., Boulinier et al., 2001), the exchange of ticks among host individuals is limited (McCoy et al., 2003). Thus each seabird species must largely maintain its own cycle if we are to account for

the variable pattern of prevalence we found among different sympatric tick races and for interspecific differences in exposure found in studies of host immunology (Staszewski, 2007). More generally, the fact that we found no association between different isolates and different tick races could mean one of the two things: (1) all isolates circulate among seabirds and their associated ticks, increasing the potential to spread different genospecies at large spatial scales or, (2) we did not have the power to effectively test this possibility with the gene used. Future work should shed light on these two hypotheses (see below).

Past studies of the marine enzootic cycle have revealed the presence of only *B. garinii* and a relatively low diversity of strains (Olsen et al., 1995; Bunikis et al., 1996). Here, we found a diverse clade associated with this genospecies (Fig. 2, group 2), a clade that accounted for almost 84% of sequenced isolates. In addition to *B. garinii*, we also detected two new genospecies in the marine cycle; a single sequence grouped with *B. burgdorferi* sensu stricto and thirteen sequences were most closely related to *B. lusitaniae* (Fig. 2, group 1). *B. burgdorferi* sensu stricto represents a relatively small fraction of infections in Western Europe (Gern and Humair, 2002), but is the main genospecies present in North America. It is considered to be a generalist in terms of the vertebrate host that it can infect (Hanincova et al., 2006). In contrast, the distribution and host range of *B. lusitaniae* seems to be more limited. This genospecies has been detected as far north as Poland and, although occasionally found in passerine birds, is typically associated with lizards (e.g., Wodecka and Skotarczak, 2005; Majlathova et al., 2006). In North Africa, *B. lusitaniae* represents 96–100% of infections in *Ixodes ricinus* (Younsi et al., 2005). With the data currently available, we are unable to say for now whether the isolates found in the present study are identical to those found in terrestrial systems, or whether they represent novel groups closely related to these genospecies. More detailed studies of other housekeeping genes (i.e., multi-locus sequence, or MLS analysis; Richter et al., 2006) will now be required to determine the true extent of diversity present in this system.

Only two previous studies have surveyed the prevalence and genetic diversity of LB spirochetes in the marine system (Olsen et al., 1995; Bunikis et al., 1996). In Olsen et al. (1995), 523 ticks were tested at the *flaB* gene by PCR amplification. These ticks were sampled from nine colonies in both the Northern and Southern hemispheres and included a range of host species, with different species sampled in most colonies. Of the 113 positive ticks (~22%), only 52 *Borrelia* isolates were sequenced. The investigators found six different sequences that were all more closely related to *B. garinii* than to any other LB *Borrelia* species described at that time. Larsson et al. (2007) recently confirmed the presence of 4 strains of *B. garinii* in ticks of Hornøya using the intergenic spacer region. Bunikis et al. (1996) examined LB spirochetes in four seabird colonies in the North Atlantic (2 in the Baltic Sea, 1 in the Faeroe Islands, 1 in Iceland) at three different genes (16S, ospA, ospC). They found a much lower prevalence of infection (3.6%) and three ribotypes of *B. garinii*. In the present study, we found a similar range of prevalence values, but a higher diversity of both genospecies and isolates. This could for several reasons. For example, we amplified a slightly larger portion of the *flaB* gene that only partially overlaps with that used by Olsen et al. (1995). This region may capture more of the polymorphism of the gene. Given the variable results among studies, it is also possible that the presence of LB spirochetes in this system is relatively dynamic. Over time the distribution and abundance of different isolates may have changed. Likewise, there may be temporal variation in the abundance of different isolates that could be linked to environmental variation. In particular, seabirds are long-lived and can experience annual variation in resource availability (Furness and Monaghan, 1987). In years when resources are scarce, individual birds may be submitted to greater stress and spirochetemia may be higher (Gylfe et al., 2000), thereby increasing the infection rate of feeding ticks. In years when food resources are abundant, seabirds may effectively reduce both the number of circulating bacteria and their susceptibility to tick feeding, lowering prevalence in the local tick population. In this sense, it would be interesting to monitor the change in the prevalence of LB spirochetes present in ticks and seabirds within given colonies over time.

Here, we analyzed *Borrelia* diversity using a relatively well-conserved housekeeping gene (*flaB*) that revealed the presence of three genospecies circulating in marine birds, two strongly pathogenic to humans and the other a suspected pathogen (Collares-Pereira et al., 2004). However, the pathogenicity of LB spirochetes is typically associated with their ospC genotype, with most variants found in nature being non-infectious or non-pathogenic for humans (Baranton et al., 2001). Although clinical disease in humans has already been associated with the marine cycle (Gylfe et al., 1999), the characterization of ospC diversity during MLS analysis would enable us to make some direct conclusions about the role of seabirds as disease reservoirs. However, regardless of the frequency of human disease causing isolates, the marine cycle may still represent an important source of novel genetic variants that could feed into terrestrial cycles. The combined analysis of genetic variation in terrestrial and marine cycles would help us determine the potential frequency of transfer events between these two presumably independent cycles (Qiu et al., 2004).

In this study, we found a high prevalence and diversity of LB spirochetes in the seabird tick *I. uriae*. Although many alternative vector species are sometimes associated with specific LB genospecies (Wang et al., 1999), *I. uriae* does not appear to be restricted to the transmission of only *B. garinii*. We also found that the prevalence of *Borrelia* infection tended to vary among different geographic locations and among host species. The inclusion of new seabird host species and colonies from across the range of *I. uriae*, including Pacific and Southern hemisphere populations, will now be required to improve our understanding of the spatial dynamics of LB spirochetes in marine birds. For now, it is unclear where and how frequently contact occurs between terrestrial and marine LB cycles. Areas where species like *I. ricinus* or *I. persculatus* co-occur with *I. uriae* represent possible contact zones and should be evaluated in future studies (Olsen et al., 1993; Bunikis et al., 1996). Clearly, the fact that three potentially pathogenic genospecies have now been found in the marine system suggests that seabirds may indeed play an important role in Lyme disease epidemiology.

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