

LOCAL DISTRIBUTION AND GENETIC STRUCTURE OF TICK-BORNE PATHOGENS: AN EXAMPLE INVOLVING THE MARINE CYCLE OF LYME DISEASE

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Abstract

Despite the potential importance of the local structure of micropathogen populations for the epidemiology of vector-borne diseases, the spatial and temporal heterogeneity of these populations is often neglected. This variability may have strong effects on micropathogen transmission, and therefore needs to be considered more explicitly to understand disease dynamics. Here, we examine the effects of time (years) and space (cliffs) on the local distribution and genetic structure of Lyme disease bacteria *Borrelia burgdorferi* sensu lato in the marine system involving seabirds and the tick *Ixodes uriae*. We tested for the presence of *Borrelia* spp. in 351 ticks collected from Black-legged kittiwakes (*Rissa tridactyla*) within a large seabird colony by amplification of the *flaB* gene. Overall, the prevalence was 11% ($\pm 2\%$) and varied among sub-colonies (i.e., cliffs), but not among years. Direct sequencing of the amplified products revealed the presence of three species of *Borrelia burgdorferi* s.l.: *B. garinii*, *B. burgdorferi* sensu stricto and *B. afzelii*. This is the first record of *B. afzelii* in the marine system. Isolates of *B. garinii*, the most abundant bacterial species, were genetically structured among cliffs, but did not change significantly over time. Our findings indicate that LB spirochetes circulating in the marine cycle are highly diverse, even at a local scale, and that the spatial structure revealed by our data should be considered more widely in this system and in epidemiological studies of Lyme disease in general. For example, adequate sampling designs to reliably estimate parameters such as local prevalence, abundance and diversity will need to take this heterogeneity into account.

Key-words:

Infectious disease, micropathogen population structure, colonial seabirds, *Ixodes uriae*, *Borrelia burgdorferi* sensu lato

Introduction

In epidemiological studies pathogen populations are often treated as being unstructured at the scale of the host population. However, micropathogen populations may vary both spatially and temporally, even at relatively small spatial scales (e.g., Wood *et al.* 2007) and this structure can have important implications for local transmission dynamics, the evolution of these pathogens, and our perception of disease risk.

Indeed, due to their ability to trigger sudden epidemics and their potential for rapid evolution, parasites and emerging infectious diseases have become a major focus in ecology and evolution and the importance of integrating their dynamics into epidemiological modelling is becoming increasingly apparent (Levin *et al.* 1999).

The spatial environment of parasites is structured at two ecologically different spatial levels: the host (biotic environment) and the habitat of the host

(abiotic and biotic environments) (Thomas *et al.* 2002). Both host heterogeneity (inter and intraspecific) and the spatial variability of the host's habitat (i.e., ecosystem) may represent strong constraints on parasite ecology. These factors, and the resulting infection dynamics, can also vary over time. For example, periodic changes in climate or resource availability may modify interactions between hosts and parasites (i.e. encounter rates, virulence, resistance) and thus modify the probability of transmission (Altizer *et al.* 2006). As most pathogens have short generation times, large population sizes and are under relatively strong selection pressures, these types of ecological changes can accelerate micropathogen evolution (Ferguson *et al.* 2003). The spatial and temporal variation of the parasitic environment may be especially important in complex disease cycles that involve numerous host types, as is the case for vector-borne parasites. These systems are frequent in nature and often of great medical and economic interest. Indeed, about a third of micropathogens responsible for emerging infectious diseases are transmitted to humans via vectors (Jones *et al.* 2008).

Despite the recognized need for the integration of spatio-temporal dynamics to better understand the epidemiology of vector-borne disease, few field studies explicitly consider these factors and partially so at local spatial scales (but see Burdon & Thrall 1999; Bensch & Akesson 2003; Wood *et al.* 2007). This may be due in part to the fact that the integration of these aspects is not always easy. Such systems are complex by definition (e.g., several possible host types within the same habitat), sampling can be difficult and basic knowledge of the species involved may be missing. Here, we consider a biological system involving the tick, *Ixodes uriae*, and one of its colonial seabird hosts, the Black-legged kittiwake *Rissa tridactyla*. Along with other pathogens, this ectoparasite and its seabird hosts carry bacteria responsible for human Lyme disease. This system has the advantage that the populations of the host and vector are spatially discrete and seasonally predictable. In addition, much is already known about the basic biology of the organisms involved (see below). This system is therefore highly suitable for studying the spatial and temporal factors shaping local pathogen dynamics.

Lyme disease is the most commonly reported vector-borne human disease in temperate regions of the Northern hemisphere and has significant medical and economic impacts (Zhang *et al.* 2006). The causative agents are spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex. This

complex contains at least 13 species, among which four are currently described as pathogenic for humans: *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* and *B. spielmanii*. The terrestrial cycle of this disease, involving ticks of the *Ixodes ricinus* complex and a wide variety of vertebrate hosts (mammals, birds, reptiles), is relatively well studied. However, comparable information on the general importance of the marine cycle and the role of seabirds in the global epidemiology of Lyme disease are lacking.

Initially, *Borrelia garinii* was the only species described circulating in the marine system (Olsen *et al.* 1993; 1995; Gylfe *et al.* 1999). However, recently, *B. burgdorferi* sensu stricto and *B. lusitanae* have also been discovered in seabird ticks (Duneau *et al.* 2008). Although described as a seabird generalist parasite, recent genetic work has shown that *Ixodes uriae* has in fact formed distinct seabird species-specific host races and that the evolution of these races is on-going and recurrent (McCoy *et al.* 2001; McCoy *et al.* 2005). This divergence could lead to cascading host-associated genetic differentiation in *Borrelia* (McCoy *et al.* 2008). Microhabitat heterogeneity (cliff topography, temperature, hygrometry, etc), inter-individual differences in bird immunity and a limited capacity for active dispersal can also greatly affect tick ecology and potentially lead to within-colony structure of these populations (e.g., Needham & Teel 1991; Boulinier *et al.* 1996; Gylfe *et al.* 1999; McCoy *et al.* 1999; McCoy *et al.* 2003; Staszewski *et al.* 2008), and consequently of the parasites they carry. We can therefore expect variability in LB spirochetes at this spatial scale. Similarly, temporal variation in environmental factors such as temperature or resource availability can greatly affect the dynamics of seabirds (Sandvik *et al.* 2005) and possibly of their ticks (Ogden *et al.* 2006; Rosa *et al.* 2007). However, the degree to which this variability affects the transmission of pathogens like *Borrelia* spp. is unclear. In this sense, it would be interesting to monitor changes in prevalence and genetic variability of LB spirochetes present in ticks over time.

To improve our understanding of the importance of local factors that may affect pathogen transmission patterns in vector-borne systems, we analyzed the effect of time (years) and space (cliffs) on the prevalence and genetic structure of LB spirochetes within a large seabird colony. We focused our investigations on ticks sampled from the most numerous seabird host in the studied area, the Black-legged kittiwake (*Rissa tridactyla*). We discuss the ecological factors that may lead to

the observed patterns and the consequences of these results for the transmission, dynamics, and evolution of Lyme disease bacteria.

Materials and methods

1. Tick sampling

Ticks of different stages (nymphs, adult males and females) were sampled directly from kittiwakes (*Rissa tridactyla*) in a large colony on Hornøya, an island in Northern Norway (70°22'N, 31°10'E) (Figure 1).

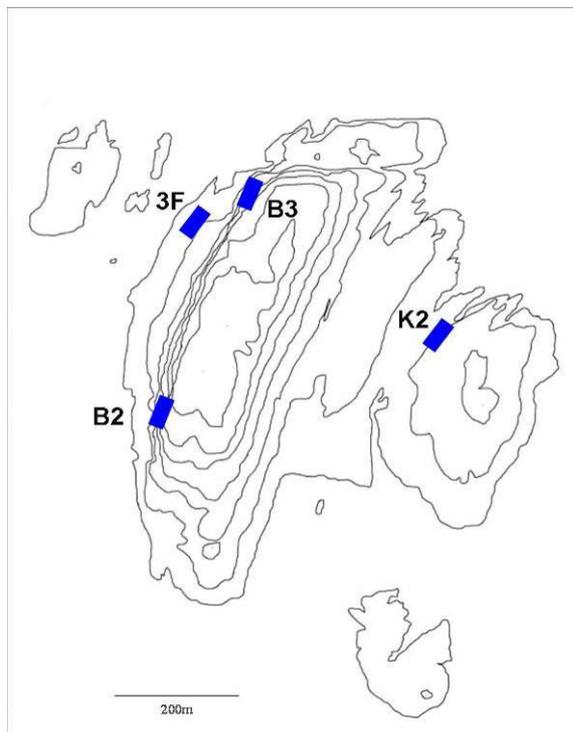


Figure 1. Location of the kittiwake cliffs sampled for *Ixodes uriae* on Hornøya in 1998 (70°22'N, 31°10'E). Approximately 15 000 pairs of kittiwakes breed in small cliffs spread across the island (Sandvik *et al.* 2005).

This colony is spatially sub-divided into discrete breeding cliffs where breeding pairs nest in groups on the vertical parts of the cliff face. Ticks can readily be collected from nestlings during the chick-rearing period (McCoy *et al.* 1999). To investigate the effect of year, we used ticks collected in 1998, 2005 and 2006 from across the island. To test the effect of cliff, we analysed kittiwake ticks collected in 1998 from birds found in different cliffs (K2, B2, B3 and 3F; Figure 1). An effort was made to collect at least 40 ticks for each cliff (K2, B2, B3 and 3F) and each year of the study (1998, 2005 and 2006). Within each cliff, the majority of ticks were collected in different nests.

After collection, ticks were stored in 70-90% ethanol until DNA extraction.

2. DNA extractions, nested PCR 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. A steel bead was then added in a 1.5ml 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 100µl 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 species of *B. burgdorferi* sensu lato (Johnson *et al.* 1992). This gene is located on the linear chromosome of the bacterium and encodes a 41kDa flagellin protein. During the first PCR, a 611pb portion of the gene was amplified with primers outer1 and outer2 (Johnson *et al.* 1992). A small amount (0.5µl) 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 390pb sequence of the polymorphic region of this gene (Gassman *et al.* 1989). Each 25ml reaction mixture was composed of 2.5ml 10x buffer (Tris-HCl, pH 9.0, KCl, Triton1 X-100), 2ml MgCl₂ (25 mM), 2ml dNTP (2.5mM), 0.5ml forward primer (20mM), 0.5ml reverse primer (20mM), 1.25U Taq polymerase (Promega), 20-50ng DNA and sterile, distilled water. The PCR conditions consisted of an initial denaturation step at 95°C for 1min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40s, and extension at 72°C for 1min, with a final extension at 72°C for 5min. The second PCR followed the same program, but the annealing temperature was 55°C. All PCRs were run with positive (DNA from cultured *B. garinii*-20047) and negative (distilled water) controls. A 390pb band on a 2% agarose gel was used as an indication of the presence of LB spirochetes in the sample. All positive amplifications were re-amplified and sent for direct sequencing (Genome Express, Meylan France). To increase the quality of sequences, both DNA strands (forward and reverse) were sequenced. All negative samples were tested at least twice.

3. Analyses of diversity and spatial and temporal structure

In order to describe the diversity and relationship of the detected LB spirochetes strains, we carried out a phylogenetic analysis. The freeware BIOEDIT (Hall 1999) was used to verify the complementarity of the *flaB* sequences obtained. We aligned our sequences with 24 reference sequences obtained from Genbank. Reference sequences included two divergent sequences from each of 13 currently described species of the *B. burgdorferi* s.l. complex, if available, and a *B. hermsii* sequence (relapsing fever *Borrelia*) used as the outgroup (e.g., Fukunaga *et al.* 1996) (Table 1).

Table 1. Reference sequences used in the phylogenetic analyses.

Species	Isolate	Genbank accession no.
<i>Borrelia afzelii</i>	Iper 3	AY342020
	ACA1	AB035613
<i>Borrelia andersonii</i>	21038	D83763
	19857	D83762
<i>Borrelia bissettii</i>	CA128	DQ393343
<i>Borrelia burgdorferi</i> sensu stricto	B31	X15661
	GeHo	X15660
<i>Borrelia californiensis</i>	CA443	DQ393348
	CA404	DQ393346
<i>Borrelia garinii</i>	Ip90	L42885
	20047	D82846
<i>Borrelia japonica</i>	NT112	D82853
	HO14	D82852
<i>Borrelia lusitaniae</i>	47ZLIM	DQ788619
	D23-04	DQ016623
<i>Borrelia sinica</i>	CMN1a	AB022138
	CMN3	AB022138
<i>Borrelia spielmanii</i>	PC-Eq2/1	AY450560
<i>Borrelia tanukii</i>	Hk501	D82847
	OR1eR	D85070
<i>Borrelia turdi</i>	Kt501	D82851
<i>Borrelia valaisiana</i>	OS66/01	AB091715
	CMN1b	AB022134
<i>Borrelia hermsii</i> (outgroup)	YOR	AY597806

We used MODELTEST 3.7 to search for the best-fit model of nucleotide substitution for our sequence data (Posada & Crandall 1998). The selected model was then applied in a maximum likelihood phylogenetic analysis using PHYML (Guindon & Gascuel 2003) and the resulting tree was drawn using TREEVIEW (Page 1998). Bootstrap analysis (1

000 repetitions) was performed to evaluate the robustness of branches.

Differences in prevalence of *Borrelia* positive ticks among years and cliffs were tested using Fisher's exact tests with the program STRUC of the software GENEPOP (Raymond & Rousset 1995). We then tested whether these isolates showed spatial or temporal genetic structure. AMOVA analyses were performed among years and among cliffs using ARLEQUIN 3.1 (Excoffier *et al.* 2005). The significance of the estimated variance components was tested by a permutation procedure.

Results

Among the 351 ticks tested, we found 38 positive for *B. burgdorferi* s.l. The bacterium was found in ticks of the 3 years and in the 4 cliffs considered (Table 2).

1. Phylogenetic analyses

Among the 38 positive samples, 29 clear sequences were obtained and we found 14 different haplotypes. For the phylogenetic analysis, we therefore used the 14 sequences from our samples and the 24 references sequences from Genbank. A given sequence was only included once in tree construction to avoid giving disproportionate weight to certain mutational events. After alignment, a 367pb region of the *flaB* gene was used for the analysis. The sequences from the present study clustered into three well-supported groups (Figure 2). The majority of our samples (72%) clustered with the *B. garinii* reference sequences (Group 1). Seven sequences (24%) grouped close to *B. burgdorferi* s.s (Group 2), and one sequence (4%) was most closely related to *B. afzelii* (Group 3).

2. Prevalence

The overall prevalence across years and cliffs was 11% (\pm 2%). There was no difference in the prevalence of infected ticks among the three years (1998, 2005 and 2006; Fisher's exact test, $p=0.360$; Figure 3a). However, the time step between years 2005 and 2006 may not represent independent samples given that a single tick generation takes approximately 4 years to complete. We therefore also grouped these two years and tested for a difference between 1998 and 2005/2006: no significant difference was found in this time period (Fisher's exact test, $p=0.280$). However, prevalence did vary significantly among cliffs (Fisher's exact test, $p<0.05$). The prevalence from ticks collected

in cliffs B2, B3 and 3F was similar and significantly higher than that of cliff K2 (Figure 3b).

Table 2. Prevalence of LB spirochetes in kittiwake *Ixodes uriae* ticks from Hornøya.

	1998	2005	2006	Total
No. ticks	233	57	61	351
(N, AM, AF)	(110, 0, 123)	(0, 35, 22)	(2, 0, 59)	(112, 35, 204)
No. positive ticks	22	9	7	38
(N, AM, AF)	(15, -, 7)	(-, 9, 0)	(0, -, 7)	(15, 9, 14)
Prevalence (%)	9	15	11	11
(N, AM, AF)	(14, -, 6)	(-, 26, 0)	(0, -, 12)	(13, 26, 7)

* N : nymph; MA : adult male; AF : adult female

3. Genetic structure

Given that *B. garinii* was the most common species found in ticks (72%), we excluded *B. burgdorferi s.s* and *B. afzelii* from the following analysis to avoid bias induced by the presence of highly divergent *Borrelia* species. We did not performed independent analysis for this species because of their low prevalence. We found that *B. garinii* isolates were not genetically structured in time (1998, 2005, 2006; AMOVA, $F_{st} = -0.322$, $p = 0.936$ and 1998 vs. 2005/2006; AMOVA, $F_{st} = -0.308$, $p = 0.935$). For the spatial analysis, we excluded cliff K2 because only one sequence was available for this cliff. We found significant spatial genetic structure among cliffs. *B. garinii* isolates from cliff 3F were genetically different from isolates of the two other cliffs (AMOVA, $\Phi_{ST(3F,B2)} = 0.628$, $p < 0.001$; $\Phi_{ST(3F,B3)} = 0.626$, $p < 0.001$).

Discussion

Despite almost two decades of research focused on understanding the global epidemiology of Lyme disease, we still lack key elements on the local structure of host, vector and micropathogen populations and how these structures may alter transmission patterns. We also are missing information on the relative importance of alternative transmission cycles, such as the role of seabirds in the maintenance and transmission of LB spirochetes. Here, we focused on the structure of the micropathogen population at a fine spatial scale. We analyzed the prevalence and genetic structure of LB spirochetes in kittiwake ticks to examine the factors that may affect local transmission dynamics and to consider the

potential importance of such variability for epidemiological studies. We found a global prevalence of 11% ($\pm 2\%$) and detected the presence of three species of LB spirochetes, one of which had never been previously detected in the marine cycle.

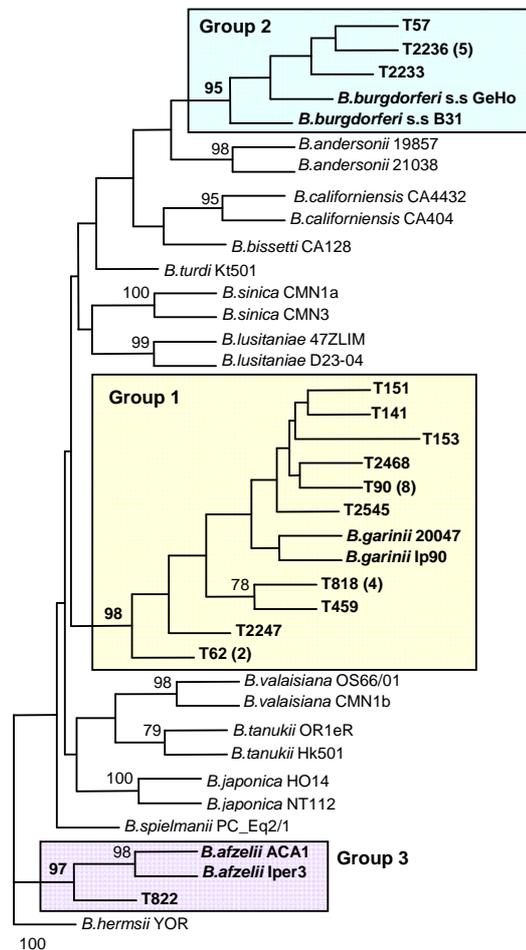


Figure 2. Maximum likelihood phylogenetic tree of the *Borrelia flaB* sequences. Only bootstrap values greater than 75% are indicated on the tree. The sequences from this study are referred to by a "T" followed by a number. Several copies of a given sequence were sometimes found; the total number is indicated in brackets. Sequences clustered in three well-supported groups: group 1 is closely related to *B. garinii*, group 2 clustered with *B. burgdorferi s.s* and group 3 is related to *B. afzelii*.

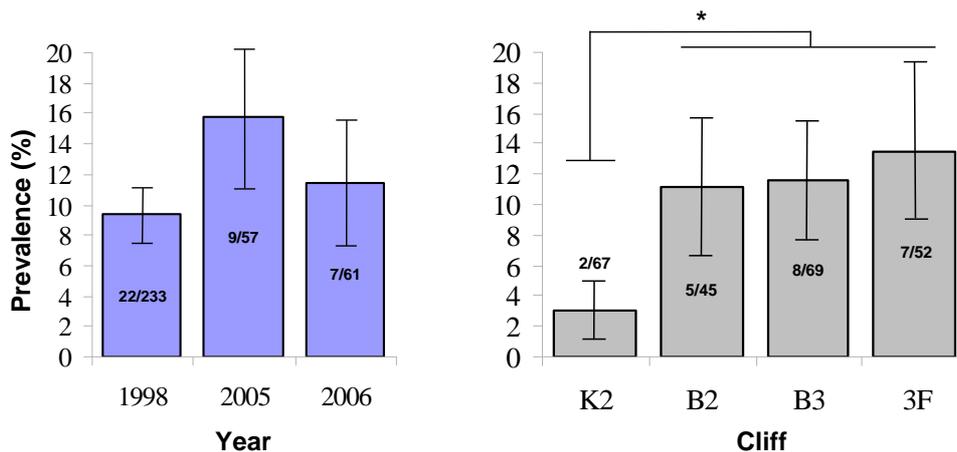


Figure 3. Prevalence of *B. burgdorferi* s.l. in the tick *Ixodes uriae*. (a left) Among kittiwake ticks collected in different years (b right) Among kittiwake ticks collected in different cliffs within the same year (1998); the symbol (*) means $p < 0,05$. The confidence interval of the prevalence was computed by calculating the standard deviation of binomial law $\sqrt{((p(1-p))/n)}$ where p is the prevalence and n the total number of analyzed ticks. The number of the positive ticks over the number tested is indicated on the histogram.

Diversity of LB spirochetes in the marine cycle

In this study, we identified three species of LB spirochetes belonging to the *Borrelia burgdorferi* s.l complex. The most abundant species, *B. garinii* was previously described in seabird ticks of both hemispheres (Olsen *et al.* 1993; 1995). This bacterial species seems to be the dominant species in the marine system and shows relatively high polymorphism (Lagal *et al.* 2002; Duneau *et al.* 2008). We also detected *B. burgdorferi* s.s, a species which has been detected once before in the same colony in a tick sampled from a common guillemot (*Uria aalge*) (Duneau *et al.* 2008). It should be noted that we have also found this bacterial species in further recent analyses of puffin ticks on Hornøya (data not shown). In the terrestrial cycle, *Borrelia burgdorferi* s.s is considered to be a generalist in terms of the vertebrate host that it can infect (Hanincova *et al.* 2006). Our results from kittiwake ticks support the presence of *B. burgdorferi* s.s in the marine cycle. Interestingly, we also found *B. afzelli*, a species which has never been previously described in seabird tick. We have also found this bacterial species in puffin and guillemot ticks on Hornøya (data not shown). The question of a link between terrestrial and marine cycles therefore requires further consideration as several bacterial species seem to be shared between these supposedly independent cycles.

In this study, we did not detect *B. lusitaniae*, another species recently found in *Ixodes uriae* in Iceland (Duneau *et al.* 2008). As the prevalence of

B. garinii isolates varies between geographic sites in the North Atlantic (Duneau *et al.* 2008), and *B. burgdorferi* s.s and *B. afzelli* have not yet been found in ticks from colonies other than Hornøya, this could suggest that the circulation of some strains is constrained geographically. This observation is in agreement with the genetic structure found in the tick vectors of this pathogen, where populations in Northern Norway were found to be strongly differentiated from other North Atlantic colonies (McCoy *et al.* 2005). It should nevertheless be noted that a full picture of the circulation of the bacterial strains in the marine cycle will require further sampling and analyses in other large colonies before any generalization can be made. Nevertheless, we can say our results support the hypothesis that seabirds are avian reservoirs of LB spirochetes and that the dispersal abilities of birds (seabirds, migratory passerines) could be an important element in the transmission of these pathogens at large spatial scales (Olsen *et al.* 1993; Olsen *et al.* 1995; Larsson *et al.* 2007; Duneau *et al.* 2008).

Temporal dynamics

A consideration of temporal infection dynamics is required to understand the interactions among the different species implicated in vector systems. Periods of high microorganism transmission can alternate with periods of low transmission, sometimes leading to the local extinction of microorganism populations (e.g., Craig *et al.* 2004). For instance, in the case of marine cycle of Lyme disease, birds may be submitted to greater stress

and spirochetemia may be higher (Gylfe *et al.* 2000) in years when resources are scarce, thereby increasing the infection rate of feeding ticks. In years when resources are abundant, tick infestation and LB spirochete transmission could decrease because the immune response of birds is more effective at reducing circulating bacteria. In the same way, annual changes in climatic conditions (temperature, humidity, etc) can affect the number of ticks likely to transmit LB spirochetes (Ogden *et al.* 2006). By limiting the population size of the vector, such fluctuations can modify the genetic diversity of micropathogens and lead to rapid genetic drift (Ferguson *et al.* 2003).

In our study, prevalence did not vary in time and *B. garinii* isolates were not genetically structured between years. This suggests that the epidemiological cycle of LB spirochetes at the study site is relatively stable over time. However, these results should be treated with caution with respect to the time scale considered here. A time step of eight years may not be long enough to detect molecular divergence in isolates, and particularly so with the conserved gene we considered here. Likewise, the number of years analyzed may not be enough to observe a difference in prevalence. Nevertheless, different hypotheses could explain these preliminary results. First, the environmental conditions may be constant enough in time (e.g. climate, fish resources for birds) to stabilise transmission rates. Recent work has shown an interannual repeatability in seabird immunity against LB spirochetes (Staszewski *et al.* 2007). This could be due to repeated exposure to the bacteria or to the persistence of LB spirochetes in infected birds. Indeed, *Borrelia burgdorferi* s.l can persist in birds over several months (Isogai *et al.* 1994; Olsen *et al.* 1996) and parasitemia can be reactivated in periods of stress (Gylfe *et al.* 1999). The temporal patterns observed may also depend on when tick infection takes place. If infections in birds are reactivated after migration to the breeding site, effects such as resource availability at the breeding site and adult body condition may not come into play in infection dynamics (i.e., vectors feed on birds before these effects are seen).

Spatial structure

Habitat heterogeneity has been studied in detail in the terrestrial cycle of Lyme disease (e.g., Van Buskirk & Ostfeld 1998; Medlock *et al.* 2008). Van Buskirk & Ostfeld (1998) have shown, for example, that spatial variability may have an important effect on the distribution of ticks and the

prevalence of LB spirochetes. In the marine cycle, the distribution of *Ixodes uriae* among hosts has been found to vary at different hierarchical scales (Bouliner *et al.* 1996). In the Southern hemisphere, among different parts of a King penguin (*Aptenodytes patagonicus*) colony, ticks were found to be more abundant in dry habitats with shelter rocks compared to more humid areas of the colony (Gauthier-Clerc *et al.* 1998, 1999). Therefore, habitat structure is likely an important factor shaping the local distribution of ticks and thus the transmission probability of LB spirochetes.

In our study, prevalence varied at a small spatial scale; LB spirochetes were more abundant in ticks from cliffs on the west side of the island (cliffs B2, B3 and 3F) compared to the east side (cliff K2). Moreover, we observed genetic structure of *B. garinii* isolates among cliffs. These results could be explained by both abiotic and biotic factors. For example, cliff topography may play a role in the ability of ticks to disperse locally (McCoy *et al.* 2003). Given that seabirds are highly faithful to their nest sites among years (Danchin *et al.* 1998), ticks may be more or less isolated within a cliff depending on the local density of nests. Likewise, a recent study has shown that the specific immunity of seabirds against LB spirochetes can also vary between cliffs (Staszewski *et al.* 2008). Combined with the spatial stability of these birds and their nest parasites, the resulting patterns of among cliff variability in *Borrelia* spp. may be linked to differences in individual susceptibility to infection by the bacteria.

Conclusion and perspectives

Like other vector-borne disease systems, the marine cycle of Lyme disease is a complex and dynamic system (Kurtenbach *et al.* 2006); hosts, vectors and micropathogens can vary at local scales and this variability can alter the transmission patterns and, as a consequence, the distribution of disease risk. Together with previous studies on this system, our results underline the fact that the three elements implicated in vector systems have to be treated together to better understand disease epidemiology. More specifically, spatial and temporal variability in micropathogen populations will need to be considered explicitly in empirical studies of Lyme disease. For example, samples may need to be obtained from throughout a given population in order to obtain reasonable estimates of diversity and prevalence.

Here we found that *Borrelia* spp. infection in *I. uriae* ticks varies among different cliffs within a single colony. The next step will now be to link our

results with other ecological and epidemiological parameters (microhabitat conditions, tick infestation rates, bird immunocompetence and philopatry) to improve our understanding of the spatial and temporal dynamics of LB spirochetes in marine birds. Moreover, the same approach in ticks from other seabird species (e.g., puffins, guillemots) and in other colonies will be necessary to verify the general nature of our results. Finally, further genetic analyses would help us to determine how close the bacterial isolates found in this study are to those in the terrestrial cycle (see Duneau *et al.* 2008). Given that LB spirochetes in birds often show higher polymorphism than those circulating in mammals (Ras *et al.* 1997; Wang *et al.* 1999), seabirds could represent an important source a novel strains of LB spirochetes. According to the degree of interaction between terrestrial and marine Lyme disease cycles, and given the high dispersal capacity of birds, the involvement of the marine system may greater alter our current perception of Lyme disease epidemiology.

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