Strain Diversity of *Borrelia burgdorferi* in Ticks Dispersed in North America by Migratory Birds

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Strain diversity of *Borrelia burgdorferi* in ticks dispersed in North America by migratory birds

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ABSTRACT: The role of migratory birds in the dispersal of *Ixodes scapularis* ticks in the northeastern U.S. is well established and is presumed to be a major factor in the expansion of the geographic risk for Lyme disease. Population genetic studies of *B. burgdorferi* sensu stricto, the agent of Lyme disease in this region, consistently reveal the local presence of as many as 15 distinct strain types as designated by major groups of the ospC surface lipoprotein. Recent evidence suggests such strain diversity is adaptive to the diverse vertebrate hosts that maintain enzootic infection. How this strain diversity is established in emergent areas is unknown. To determine whether similar strain diversity is present in ticks imported by birds, we examined *B. burgdorferi* strains in *I. scapularis* ticks removed from migrants at an isolated island site. Tick midguts were cultured and isolates underwent DNA amplification with primers targeting ospC. Amplicons were separated by gel electrophoresis and sequenced. One hundred thirty-seven nymphal ticks obtained from 68 birds resulted in 24 isolates of *B. burgdorferi* representing eight ospC major groups. Bird-derived ticks contain diverse strain types of *B. burgdorferi*, including strain types associated with invasive Lyme disease. Birds and the ticks that feed on them may introduce a diversity of strains of the agent of Lyme disease to emergent areas. ***Journal of Vector Ecology* 36 (1): 24-29. 2011.**

Keyword Index: Ixodes scapularis, *Borrelia burgdorferi*, strain diversity, dispersal.

INTRODUCTION

Migratory birds contribute to the dispersal of *Ixodes scapularis* in North America (Anderson et al. 1986, Smith et al. 1996, Klich et al. 1996, Scott et al. 2001, Ogden et al. 2008). Previous studies in coastal Maine document infestation in 1-2% of birds by nymphal ticks during spring migration and 0.2% by larval ticks in the fall (Smith et al. 1996). The association of ticks with particular bird species provides a means for the colonization of suitable habitats by introduced ticks, but the mechanisms for the subsequent emergence of enzootic *Borrelia burgdorferi* sensu stricto at these new sites are unknown.

In the northeastern U.S., local populations of juvenile *I. scapularis* (i.e., larvae and nymphs) feed on diverse rodent and bird hosts. In these settings, strain diversity that includes ≥8 (range 8-15) or more ospC major groups is typical (Wang et al. 1999, Qiu et al. 2002, Brisson and Dykhuizen 2004, Alghaferi et al. 2005, Anderson and Norris 2006, Hanincova et al. 2006). OspC is a surface lipoprotein of *B. burgdorferi* expressed during initial infection of vertebrate hosts, and ospC allele diversity is commonly used to characterize *B. burgdorferi* strain diversity (Brisson and Dykhuizen 2004). The maintenance of this strain diversity is potentially due to balanced selection based on adaptive advantages for particular strains in particular reservoir hosts (Qiu et al. 2002, Brisson and Dykhuizen 2004). If introduction of *B. burgdorferi* occurs primarily by dispersal of ticks from birds, and if enzootic Lyme disease is established by these introduced ticks, strain diversity typical of established areas should be present in migratory birds, the infected ticks that they carry, or both. However, prior studies indicate that birds might not serve as reservoir hosts for all strains, or that there is elimination of particular strains when ticks feed on birds (Mather et al.1989, Matuschka and Spielman 1992, Bunikis et al. 2004). Therefore, we sought to determine whether *B. burgdorferi* strain diversity representative of established enzootic populations is present in ticks removed from migratory birds.

MATERIALS AND METHODS

Appledore Island (N420 59/W700 36), located 9.7 km off the Maine-New Hampshire coast, is the site of an established bird banding program that monitors migration during spring and fall. Shrub habitat is present on most of the 33.6 hectare island, and mammals, with the exception of a small population of muskrats (*Ondatra zibethicus*) are absent. *I. scapularis* ticks, though present on migratory birds, are not established on Appledore Island, presumably because deer are required to maintain the tick's life cycle (Rand et al. 2004).

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During the spring of 2003, 329 nymphal and 114 larval *I. scapularis* ticks were removed from migratory birds of 11 species captured in mist nets at the island banding station during migration. Ticks were placed in labeled vials, one for each bird, and transported to the Vector-Borne Disease Laboratory at Maine Medical Center for identification using standard taxonomic keys (Cooley and Kohls 1945, Durden and Keirans 1996). One hundred thirty-eight ticks (137 nymphs and one larva) removed from 68 birds were cultured for *B. burgdorferi*. Over 80% of the ticks collected were found on one bird species, the common yellowthroat (*Geothlypis trichas*). Mid-guts dissected from ticks were placed in Barbour-Stoenner-Kelly (BSK) media, incubated at 34° C and examined by dark field microscopy after two-to-four weeks. DNA was extracted from positive cultures with the Quiagen MiniPrep kit (Quiagen Inc,Valencia, CA), and amplified by means of polymerase chain reaction (PCR). Primers targeting the osp C coding region (+5'-AAA GAA TAC ATT TGC GAT ATT-3' and -5'-GGG CTT GTA AGC TCT TTA ACT G-3') yielded a roughly 600 base pair product (Wang et al. 1999). Negative controls were included in each run.

DNA presence was confirmed for the diagnostic 600 bp PCR product by gel electrophoresis using 1% agarose with ethidium bromide to illuminate DNA under UV light with a 1 kilobase ladder from GeneRuler (Fermentas, Hanover, NH). The PCR product was then purified with Quiagen QIAquick PCR prior to sequencing by standard protocol using Rhodamine ready reaction kit (Applied Biosystems Foster City, CA) with 8 µl ready mix, 1 µl of 20 mM (+) strand primer, and 7-1 µl of purified DNA and water to bring the reaction mix to 20 µl. Samples were amplified by PCR according to manufacturer's recommendations and precipitated in ethanol, resuspended first in 3 M sodium acetate (pH 5.2), then in 20µl template suppression buffer (Applied Biosystems) and boiled for 4 min prior to sequencing using an automated (ABI 310) sequencer.

DNA sequence data were aligned using Clustal X (Thompson et al. 1997) and then adjusted using Se-Al software (Rambaut 2002). Ambiguous DNA sequence data were repeated. If, after resequencing, the chromatogram remained ambiguous, the sequence was excluded from further analysis. Insertion or deletion of bases (Indels) that spanned more than one base pair position and that differed between the ingroup and outgroup (*Borrelia garinii*) were recorded as one character for the whole gap rather than one character for each base pair position deleted. Phylogenetic analyses were carried out on the dataset, which included an additional 21 sequences that represented known ospC major groups listed on GenBank. We performed 500 rapid bootstrap inferences to assess nodal support followed by a thorough maximum-likelihood (ML) search in RAxML version 7.0.4 (Stamatakis 2006, Stamatakis et al. 2008) as implemented on the CIPRES portal (Miller et al. 2009). The ML search used a GTR substitution matrix with empirically determined base frequencies, and among-site rates were assumed to follow a gamma distribution (alpha=0.572811). All phylogenetic trees were rooted using *B. garinii* as an outgroup.

RESULTS

Twenty-four *B. burgdorferi* isolates were obtained and sequenced from twenty birds, all but two of which were common yellowthroats. No DNA was detected in negative controls. A total of approximately 525 bp encompassing the ospC coding region were sequenced for each individual, the alignment including indels was 575 bp long, and the final sequence alignment was 534 bp long after gap recording (see above). Nine of the sequences were excluded from further analysis due to ambiguities on the chromatogram that suggested the presence of more than a single strain. The final dataset, including the 21 representative ospC sequences from GenBank, and the *B. garinii* outgroup, comprised 37 haplotypes. Sixty-three percent (335 sites) of nucleotides were variable. Within the ingroup taxa, the maximum divergence was 26.6% and the divergences of the ingroup taxa from outgroup ranged from 17.5 to 25.9%. Maximum divergence within the best match ospC group varied from 0% to 6%. Inclusion in a defined osp C group required >90% identity of the ospC allele. The best ML tree based on the dataset was considerably well resolved and supported (Figure 1). Eight distinct ospC groups were represented among the 15 sequences (Table 1).

Two ticks removed from each of four individuals of one bird species (*G. trichas*) were tested, but in only one of these cases did both ticks removed from the same bird produce the same ospC strain type. The common strain types identified (ospC strains A, B) in this study represent commonly identified strains at sites with established enzootic *B. burgdorferi* in the northeastern United States.

Table 1. OspC major groups represented in isolates of *B. burgdorferi* sensu stricto from bird-derived ticks. Appledore Island, ME, 2003. (n=14).

ospC group	No. of isolates (%)
A	4 (27)
B	4 (27)
G	2 (13)
H	1 (07)
I	1 (07)
K	1 (07)
N	1 (07)
T	1 (07)

DISCUSSION

Dispersal of *I. scapularis* by migrating passerine birds is well established as a means for the expansion of the tick's range (Anderson et al. 1986, Klich et al. 1996, Smith et al. 1996). Introduction of *B. burgdorferi* infection into a newly colonized area may not occur unless infected larvae

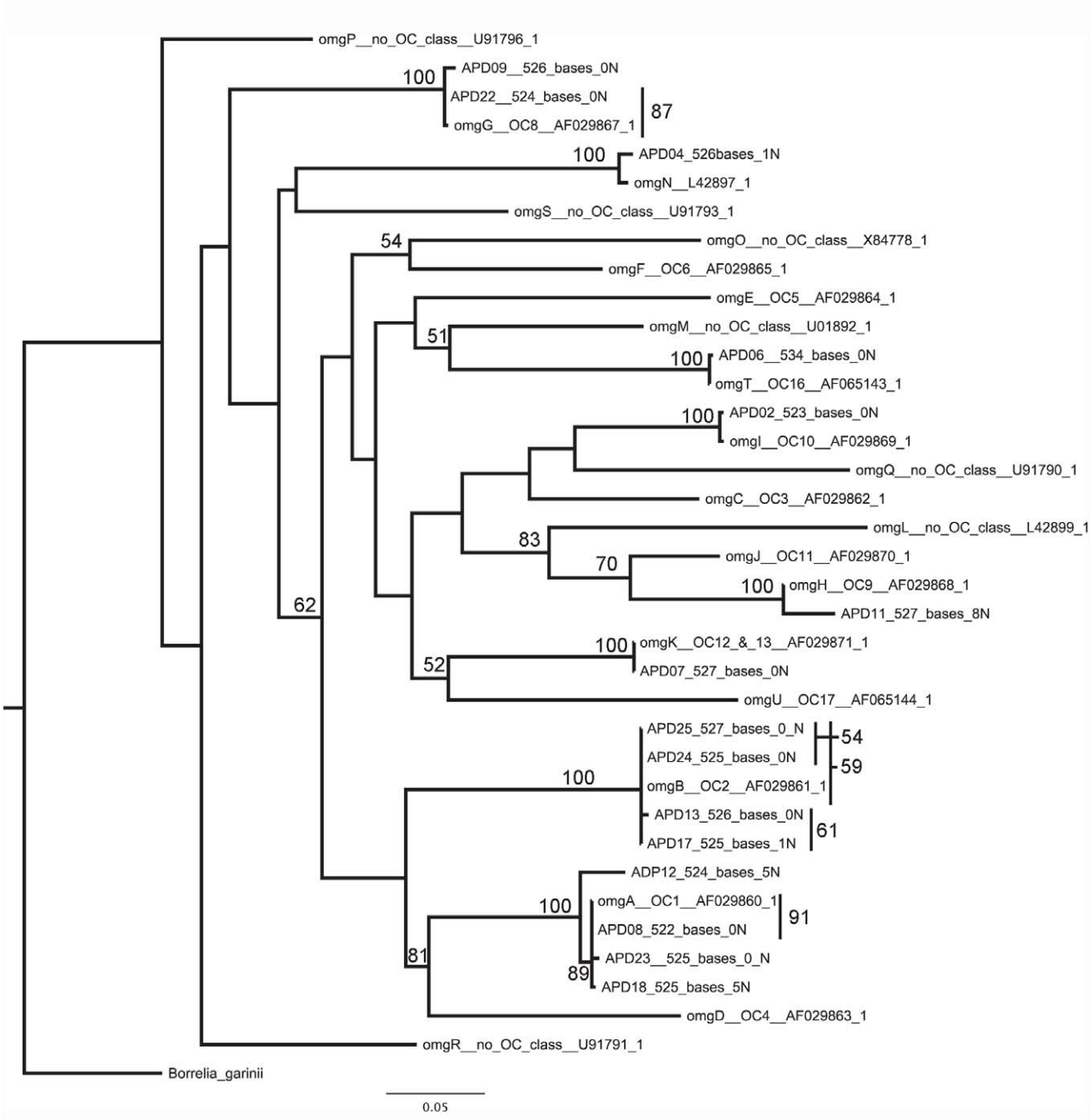

Figure 1. Best maximum-likelihood trees for 15 unique isolates from ticks removed from birds on Appledore Island (ADP#), 21 representative omG sequences from GenBank, and one outgroup (*B. garinii*). The tips of the tree are labeled with isolate identification number, the omG strains from GenBank. Branch lengths are proportional to the number of substitutions per site. Bootstrap values for maximum-likelihood (500 replicates) are reported above branches.

are present and capable of infecting additional hosts after molting into nymphs. In the northern limit of the range of *I. scapularis*, only a small fraction of ticks imported by migratory birds are larvae and the prevalence of infection with *B. burgdorferi* is low. Therefore, the capacity of birds to serve as reservoir hosts may be a requirement for the introduction of *B. burgdorferi* into areas that are geographically remote from areas already endemic for Lyme disease. The possibility that *B. burgdorferi* already exists in these emergent areas with "silent" enzootic transmission by ticks such as *I. muris* has not been documented in the northeastern U.S.

Recent reports from Europe document a role for migratory birds as reservoir hosts for *B. burgdorferi* sensu lato, though most often with the genospecies *B. garinii* (Comstedt et al. 2006). Although *B. burgdorferi* s.s. is largely maintained by rodents in the U.S., several North American bird species appear to be reservoir competent for *B. burgdorferi* s.s., and may account for the introduction of the bacterial enzootic once the tick vector is established (Rand et al. 1998, Richter et al. 2000, Ginsberg et al. 2005). Although the reservoir potential of bird species to harbor *B. burgdorferi* varies from species to species (Mather et al. 1989, Rand et al. 1998, Richter et al. 2000, Ginsberg et al. 2005), one study in Europe documented elimination of *B. burgdorferi* from ticks feeding on blackbirds (*Turdus merula*) by bactericidal activity in bird blood (Matuschka and Spielman 1992), and another study documented rapid decline of reservoir capacity in infected American robins (*Turdus migratorius*) over one-to-three months (Richter et al. 2000). Although it has not been demonstrated in *B. burgdorferi* s.s., some genospecies in Europe appear to be resistant to the bactericidal effects of complement in birds while others are sensitive (Kurtenbach et al. 2002).

In this study of ticks removed from migratory birds, we demonstrate the presence of the majority of ospC major groups of *B. burgdorferi* s.s. recognized in the enzootic sites of coastal Maine (A.M. unpublished data). As our study site is an isolated island that lacks deer and does not support completion of the life cycle of *I. scapularis*, we can conclude that these ticks carrying diverse strains of B. *burgdorferi* s.s. are dispersed by birds over long distances during migration. In established local populations, 8-15 ospC groups are present, and within-group genetic diversity of ospC is minimal (Wang et al. 1999, Brisson and Dykhuizen 2004, Anderson and Norris 2006). Nearly all strains from an established location are represented by known ospC groups and intra-group diversity is often <1% (Qiu et al. 2002). In our sample, which is presumably derived from multiple geographic regions, ospC-group diversity appears greater, perhaps reflecting a greater genetic heterogeneity within groups of strains derived from different geographic locations. For this reason, we characterized strain diversity by both inclusion (>90% sequence identity) in a major group and by maximum likelihood tree. The heterogeneity of strains present on ticks from migrating birds reported here was also noted in a recent study conducted on ticks parasitizing migratory birds in Ontario, Quebec and Prince Edward Island (Ogden et al. 2008).

Our reliance on culturing *B. burgdorferi* might limit our ability to isolate all strains present, so our results may underestimate actual strain diversity in bird-transported ticks. As we did not directly sample birds to determine whether they were infected by *B. burgdorferi,* and as the nymphal ticks we examined may acquire infection from either a mammal host when feeding as larvae or from their bird host, we cannot assume that birds serve as competent hosts for the ospC strains detected. Our number of isolates is not large enough to determine whether specific *B. burgdorferi* strains are preferentially dispersed in bird-derived ticks. The most common strain types present in this study (ospC groups A, B) are prevalent in established sites, and are associated with 'invasive" Lyme disease in humans (Seinost et al 1999, Brisson and Dykhuizen 2006). One strain of ospC (osp C group A) is hypothesized to have dispersed relatively recently and to have contributed disproportionately to the rise in Lyme disease incidence (Qiu et al. 2008).

The phylogeography of *B. burgdorferi* in the eastern United States suggests the presence of the spirochete for thousands of years prior to the recent expansion of *B. burgdorferi* out of separate foci in the Northeast and Midwest (Gatewood-Hoen et al. 2009). If some strain types are preferentially maintained in birds or the ticks they host, they might predominate in newly emergent areas. Future studies will examine strain diversity of *B. burgdorferi* present in migratory birds and further characterize the rapidity with which strain diversity emerges in areas where *B. burgdorferi* is recently introduced by birds.

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