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Genome diversity of *Borrelia garinii* in marine transmission cycles does not match host associations but reflects the strains evolutionary history

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ABSTRACT

Borrelia burgdorferi sensu lato is a species complex of spirochetal bacteria that occupy different ecological niches which is reflected in their reservoir host- and vector-associations. *Borrelia* genomes possess numerous linear and circular plasmids. Proteins encoded by plasmid genes play a major role in host- and vector-interaction and are important for *Borrelia* niche adaptation. However, the plasmid composition and therewith the gene repertoire may vary even in strains of a single species.

Borrelia garinii, one of the six human pathogenic species, is common in Europe (vector *Ixodes ricinus*), Asia (vector *Ixodes persulcatus*) and in marine birds (vector *Ixodes uriae*). For the latter, only a single culture isolate (Far04) and its genome were previously available. The genome was rather small containing only one circular and six linear plasmids with a notable absence of cp32 plasmids. To further investigate *B. garinii* from marine transmission cycles and to explore i) whether the small number of plasmids found in isolate Far04 is a common feature in *B. garinii* from marine birds and presents an adaptation to this particular niche and ii) whether there may be a correlation between genome type and host species, we initiated in vitro cultures from live *I. uriae* collected in 2017 and 2018 from marine avian hosts and their nests. Hosts included common guillemots, Atlantic Puffin, razorbill, and kittiwake.

We obtained 17 novel isolates of which 10 were sequenced using Illumina technology, one also with Pacific Bioscience technology. The 10 genomes segregated into five different genome types defined by plasmid types (based on PFam32 loci). We show that the genomes of seabird associated *B. garinii* contain fewer plasmids (6–9) than *B. garinii* from terrestrial avian species (generally ≥ 10), potentially suggesting niche adaptation. However, genome type did not match an association with the diverse avian seabird hosts investigated but matched the clonal complex they originated from, perhaps reflecting the isolates evolutionary history.

Questions that should be addressed in future studies are (i) how is plasmid diversity related to host- and/or vector adaptation; (ii) do the different seabird species differ in reservoir host competence, and (iii) can the genome types found in seabirds use terrestrial birds as reservoir hosts.

1. Introduction

Borrelia garinii is a member of the bacterial species complex *Borrelia burgdorferi* sensu lato (Kurtenbach et al., 2006) and is one of several

species causing Lyme borreliosis in humans (Stanek et al., 2010). The species prevails in natural transmission cycles involving terrestrial and marine bird species and their associated *Ixodes* vectors (Comstedt et al., 2009). It has been shown to be genetically heterogeneous across spatial

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scales and marker types (Comstedt et al., 2009; Fingerle et al., 2008; Gomez-Diaz et al., 2011; Jacquot et al., 2014; Margos et al., 2009; Masuzawa, 2004; Munro et al., 2019; Schulte-Spechtel et al., 2006; Vollmer et al., 2011). Indeed, high diversity occurs in loci that typically show relatively low diversity in other *Borrelia* species, such as the gene encoding the outer surface protein A (*ospA*) (Fingerle et al., 2008; Masuzawa, 2004; Schulte-Spechtel et al., 2006). More than seven *ospA* genotypes have been determined alone within European populations of *B. garinii* compared to only one for *B. afzelii* or *B. burgdorferi* sensu stricto. High *ospA* diversity has also been reported in *B. garinii* from Asia (Mediannikov et al., 2005). The high genetic diversity was confirmed by studies using different sets of genes such as the intergenic spacer (IGS)

between 16S and 23S rRNA (*rrs-rrf*) (Comstedt et al., 2009), multilocus sequence typing (MLST) and whole genome sequencing, where *B. garinii* shows nucleotide diversity (π) as high or even higher than *B. burgdorferi* s.s. (Becker et al., 2016; Jacquot et al., 2014; Margos et al., 2009).

Ecologically, *B. garinii* is associated with avian reservoir hosts including terrestrial birds (mainly passerines such as thrushes, tits, and robins) and seabirds such as kittiwakes, puffins, razorbills and guillemots (Comstedt et al., 2009; Comstedt et al., 2006; Comstedt et al., 2011; Dubska et al., 2009; Duneau et al., 2008; Gomez-Diaz et al., 2011; Heylen et al., 2014; Heylen et al., 2013; Norte et al., 2020; Norte et al., 2013; Olsen et al., 1993; Taragel'ova et al., 2008). A number of *Ixodes* tick species are considered vector competent for *B. garinii* including

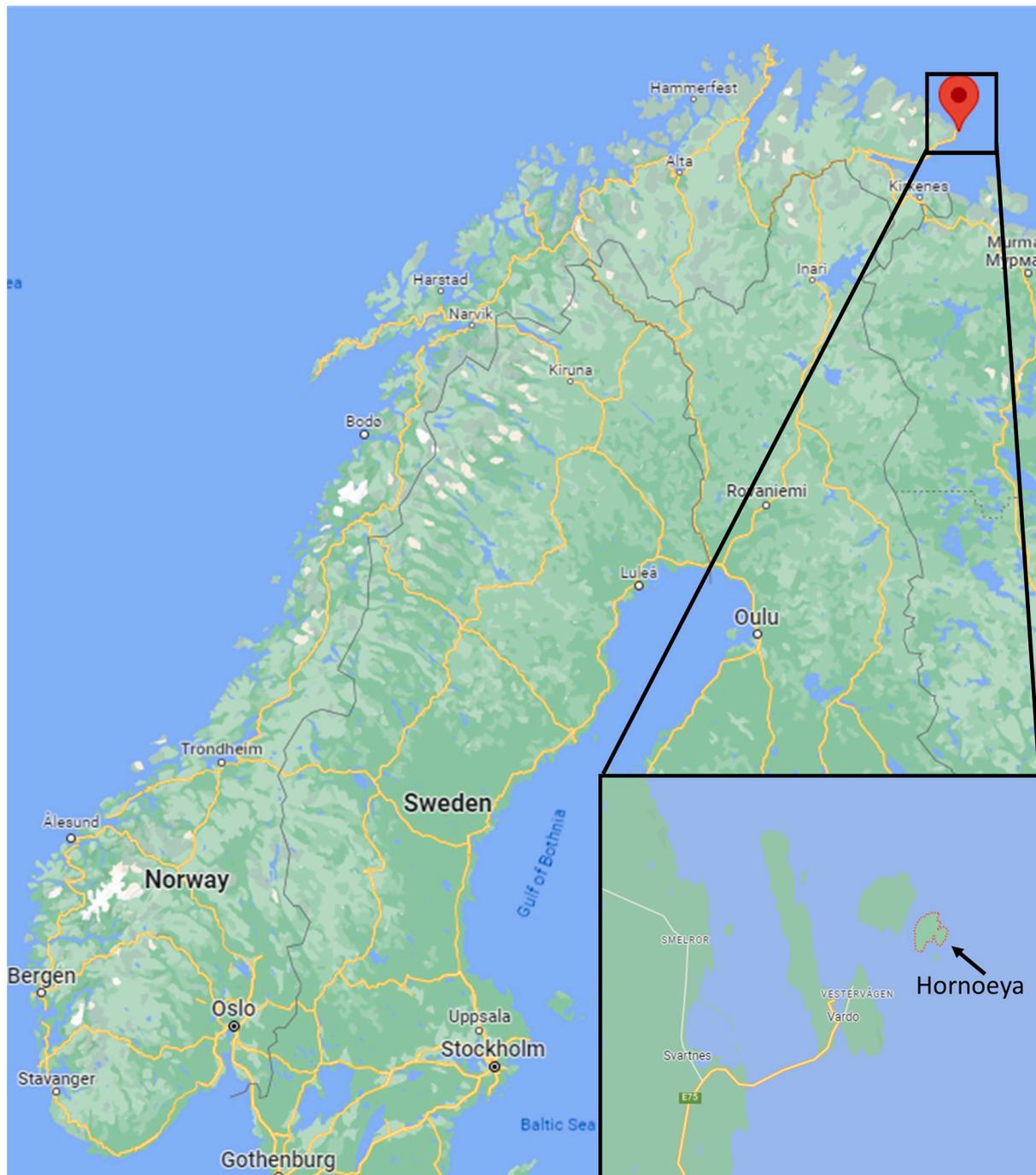


Fig. 1. Map of tick collection site in Norway. Hornøya is indicated with a red dot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Ixodes ricinus Linnaeus, 1758, *I. persulcatus* Schulze, 1930, *I. pavlovskyi* Pomerantsev, 1946 and *I. uriae* White, 1852 (Acari: Ixodidae) (Duneau et al., 2008; Eisen, 2020; Gomez-Diaz et al., 2010; Korenberg et al., 1991; Korenberg et al., 2002; Korenberg et al., 2010; Munro et al., 2019; Taragel'ova et al., 2008). In accordance with this wide spectrum of reservoir hosts and vector ticks, the geographic distribution of *B. garinii* in Eurasian temperate latitudes ranges from France to Japan in terrestrial bird-transmission cycles with *I. ricinus*, *I. persulcatus* and *I. pavlovskyi* as vectors. The distribution of *B. garinii* also extends to arctic and subarctic regions in the northern hemisphere and to subantarctic regions in the southern hemispheres, where the spirochete occurs within marine bird-tick transmission cycles involving *I. uriae* as vector (Comstedt et al., 2011; Duneau et al., 2008; Gomez-Diaz et al., 2011; Munro et al., 2019; Olsen et al., 1995; Olsen et al., 1993). A first demonstration of *B. garinii* in *I. uriae* ticks of the high Arctic (> 78° latitude) was also recently described (McCoy et al., 2022).

In vitro cultivation of seabird-associated *B. garinii* has previously been limited. Globally, there was only one isolate available obtained from an *I. uriae* tick in the Faroe Islands, designated Far04 (Gylfe et al., 1999). Sequencing of this isolate revealed a small *Borrelia* genome containing a chromosome (incomplete draft version in GenBank) and seven plasmids: cp26, lp17, lp25, lp28–9, lp36, lp32–10, and lp54 (Casjens et al., 2011). A remarkable feature was the absence of cp32 plasmids, which are found in all other *Borrelia* species (Casjens et al., 2018; Casjens et al., 2017; Schwartz et al., 2021). We hypothesized that the small genome described for Far04 may be an adaptation to marine hosts and/or vectors. In order to gain additional insights into the genome diversity of seabird-associated *B. garinii*, *I. uriae* were collected in Norway from marine hosts in 2017 and 2018. Several new cultured isolates from marine *B. garinii* were obtained and were sequenced for genome exploration.

2. Material and methods

2.1. Tick collection and processing

Ixodes uriae adult and nymphal ticks ($n = 408$) were collected in 2017 and 2018 on Hornøya, an island in northern Norway (70°23'N, 31°10'E) (Fig. 1). In 2017 (Horn17), ticks were sampled directly from seabirds or nests of the following species: Common guillemot *Uriae aalge* (Ua) ($n = 78$), Black-legged kittiwake *Rissa tridactyla* (Rt) ($n = 93$), Atlantic puffin *Fratercula arctica* (Fa) ($n = 59$) and Razorbill *Alca torda*

Table 1
Summary of *Ixodes uriae* samples included in analyses.

Host	No of birds	No of ticks	Adults	Stages Nymphs	Stage nd	No of isolates
2017						
Common guillemot <i>Uriae aalge</i>						
	13	78	50	28		5
Black-legged kittiwake <i>Rissa tridactyla</i>						
	35	93	34	59		
Atlantic puffin <i>Fratercula arctica</i>						
	16	59	4	55		8
Razorbill <i>Alca torda</i>						
	5	12	1	11		1
2018						
Off-host (OFF)*	–	124	72	37	15	3
Total	69	366	161	190	15	17

nd = not determined.

* ticks were collected off-host in a breeding colony of Atlantic puffin. Thus, it is probable that these ticks were from puffin hosts, but this is not 100% certain.

(At) ($n = 12$) (Table 1). In 2018 (Horn18), ticks were collected off-host in a breeding colony of Atlantic puffin (see Tables 1, S1 and S2 for details). As these ticks were collected on the ground, it is not 100% certain but likely that these ticks origin from puffin hosts. After collection, ticks were sent to the German National Reference Centre for Borrelia at the Bavarian Health and Food Safety Authority in Oberschleissheim, Germany for analyses. When several ticks were collected from an individual host, only a subset was analysed.

Culture. Individual live ticks were washed for three minutes in each of the following solutions: i) nuclease free water, ii) 70% ethanol and iii) phosphate-buffered saline (PBS). Following these washing steps, ticks were cut sagittally and one part was submerged in culture medium in a 96-well plate (200 µl MKP-Medium per well containing antibiotics (phosphomycin 28.6 µg/ml; sulfamethoxazole 14.3 µg/ml; amikacin 4.3 µg/ml) as described (Preac-Mursic et al., 1986; Rollins et al., 2022; Ruzic-Sabljić et al., 2006)) whilst the other half was used to extract DNA. Plates were incubated at 33 °C under a 5% CO₂ atmosphere and growth of *Borrelia* (or other microorganisms) was monitored once a week. If no *Borrelia* growth was observed by microscopy after seven to nine weeks, cultures were terminated. Cultures that showed borrelial growth were transferred into 7 ml tubes and cultured as described (Ruzic-Sabljić et al., 2014). As fully engorged ticks contained nucleated bird red blood cells, cultures had to be passaged several times until the host DNA was absent. This was a precaution as presence of host DNA may contaminate NGS library preparations and complicate downstream analyses.

2.2. DNA extraction and PCR

To isolate DNA from cultures and ticks, we used the Maxwell® 16 LEV Blood DNA Kit (Promega, Germany) according to the manufacturer's instructions. DNA of half ticks was screened for presence of *Borrelia* using a real-time PCR protocol targeting the 23S rRNA locus as described (Strube et al., 2010) on a Stratagene Mx3000P (Agilent, USA).

2.3. Multilocus sequence data analysis

Multilocus sequence typing was done according to standard procedure (see pubmlst.org/borrelia/) for details. We used PHYLOVIZ 1.1 (Francisco et al., 2012) to conduct goeBurst analyses (Francisco et al., 2009) using ST numbers, allelic profiles and meta information employing the triple locus variant (TLV) setting and to generate a minimum spanning tree. New data were submitted to the *Borrelia* MLST database (<https://pubmlst.org/borrelia/>) via the curator. Data available for North American seabird isolates ($n = 19$), Eurasian terrestrial ($n = 291$) and human ($n = 48$) *B. garinii* isolates were downloaded from the PubMLST database and included in our goeBURST analyses.

2.4. Genome sequencing and analysis

We used ten in vitro cultivated isolates to extract genomic DNA from pure cultures using the Promega Blood and tissue DNA kit (Promega, Germany). The amount of purified DNA was determined using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Germany) and adjusted to 0.2 ng/µl. Five µl were used to prepare Illumina Nextera libraries using the TG Nextera XT DNA sample preparation kit (TG-131-1096) and the TG Nextera XT Index kit (FC-131-1001) according to manufacturer's recommendations (Illumina, USA). A MiSeq® Reagent Nano Kit v2 and MiSeq® v2 reagent Kit (MS-102-2002) (Illumina, Germany) were used for sequencing. The manufacturer's protocol for library preparation was adjusted to half the volumes (Hepner et al., 2020). A library quality check was performed on a 2200 TapeStation (Agilent, High Sensitivity Tapes). For Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing of isolate 17-63N1, the Norwegian Sequencing Center (NSC) University of Oslo, Norway, used 5 µg of DNA for library preparation according to the PacBio protocol for multiplexed SMRTbell libraries using barcoded adapters. Sequencing was conducted on a

PacBio Sequel instrument using a Sequel polymerase v3.0, SMRT cells v3 LR and sequencing chemistry v.3.0.

Genome assembly of Illumina MiSeq sequences was conducted using SPAdes v. 3.11.1 (Bankevich et al., 2012). Coverage of contigs is given in Table S3. The resulting contigs were uploaded into Mauve v. 20150226 (Darling et al., 2004) for contig sorting and comparison of the newly sequenced genomes with the genomes of Far04 (GenBank accession number NC_011875.1, isolated from an *I. uriae* tick), 20047 (GenBank accession number NZ_CP028861.1, isolated from a questing *I. ricinus*) and PBr (GenBank accession number GCA_000172275.2). To concatenate chromosomal contigs the multi-draft based scaffolder MeDuSa was used (Bosi et al., 2015). PacBio read assembly was done at the NSC as part of the sequencing contract. The PacBio genome assembly of 17-63N1 was uploaded into the CLC genomics workbench v. 20 for Illumina read mapping and extraction of a consensus genome assembly. To determine the structure of plasmids (linear or circular), we employed NCBI BLASTN searches (Altschul et al., 1990). Plasmids were blasted against themselves and dot plot images provided as part of the BLAST result were used to assess whether plasmids were linear or circular; for a detailed description see (Hepner et al., 2023). To determine whether linear plasmids were complete, we identified telomere signature sequences (S. Casjens, unpublished) and whether they possessed terminal inverted repeats (wraparound) sequences following the telomere signature sequences (Table S4). Completeness of circular plasmids was determined in dot plots; contigs that had an identical overlap (terminal direct repeats) were considered complete (Hepner et al., 2023).

Plasmid typing. To determine plasmid types, we used the protein family 32 (PFam32) that was suggested as reliable marker for plasmid typing in *B. burgdorferi* s.l. (Becker et al., 2020; Schwartz et al., 2021). The locus was supposed to be homologous to the *parA* gene in *Escherichia coli* and potentially involved in plasmid replication and maintenance (Casjens et al., 2012; Stevenson and Miller, 2003).

Download of homologous sequences and phylogenetic analyses. Assembled contigs were uploaded into the Rast Annotation Server (<https://rast.nmpdr.org/>; Aziz et al., 2008). We used several genes that were previously suggested to be involved in host- or tick-interaction for comparative analyses. We included the outer surface protein A (*ospA*) which has been shown to be a ligand to a tick midgut molecule, Tick receptor for *ospA* (TropA, (Pal et al., 2004)), *ospC* which was reported to be important for vector salivary gland invasion ((Fingerle et al., 2007) or host invasion ((Grimm et al., 2004; Tilly et al., 2007)) and *cspA*, a member of the PFam54 gene array that has been shown to play a role in deflection of human and animal host complement by *Borrelia* (Kraiczky and Stevenson, 2013; Lin et al., 2020; Muhleip et al., 2018; Wywiał et al., 2009). The PFam54 gene array is located on plasmid lp54 and consists of 5 clades with clade IV being diverse even within *Borrelia* species. Apart from the function of *cspA*, the function of other genes of the gene array is unknown although they may be important in host association (Hart et al., 2018; Hart et al., 2021; Patton et al., 2013; Wywiał et al., 2009). To download homologues of genes previously suggested to be potentially involved in host and vector association (*cspA*, *ospA*, *ospC*), protein sequences were used for BLAST searches within the Rast annotation server using default settings or GenBank (see Table S5 for accession numbers of included isolates in addition to the samples from seabirds). MEGA5 (Tamura et al., 2011) was used for alignment of gene sequences and to run phylogenetic analyses. For *cspA* and its homologues, *ospA*, and *ospC*, maximum likelihood (ML) trees were generated using a general time reversible model with 500 bootstrap repeats.

2.5. Data repositories

Assembled genomes and sequence reads are available in GenBank via the following accession numbers under BioProject PRJNA327303: CP119374-CP119381 (SAMN32860459, 17-29Z1); CP118290-CP118297 genome, OQ450372, OQ450373 part of *vls* locus (SAMN32860460, 17-56Z1); CP119358-CP119365 genome, OQ450374,

OQ450375 part of *vls* locus (SAMN32860461, FNG-1Z1); CP118298-CP118305 genome, OQ450376, OQ450377 part of *vls* locus (SAMN32860462, 17-58N4); CP119383-CP119390 genome, OQ450378 part of *vls* locus (SAMN32860463, 17-59N1); CP117887-CP117893 (SAMN32860464, FNG-2Z14); CP117894-CP117901 (SAMN32860465, 17-54Z3); CP117902-CP117908 (SAMN32860466, FNG-1Z4); CP117909-CP117917 (SAMN32860467, NG-Z6); CP117839-CP117847 (SAMN32949868, 17-63N1).

3. Results

3.1. *Borrelia garinii* samples: Collection, culture and MLST

In total, 408 *I. uriae* ticks were collected in a seabird colony on Hornøya, Norway. Of these, 366 were processed at the NRZ *Borrelia*, and included 161 adults (142 females, 19 males), 190 nymphs and 15 ticks not identified to stage (Tables 1, S1). These ticks were collected from the following host species (2017) and off-host (2018): common guillemot *Uriae aalge* (Ua) ($n = 78$), black-legged kittiwake *Rissa tridactyla* (Rt) ($n = 93$), Atlantic puffin *Fratercula arctica* (Fa) ($n = 183$) and razorbill *Alca torda* (At) ($n = 12$).

From 199 of these ticks, in vitro cultures were initiated of which 17 became positive, five from ticks that were negative in qPCR (Table S2) suggesting a *Borrelia* burden below the detection limit or some PCR inhibitory substances due to blood meal residues in these ticks. Preferentially, cultures with low passage numbers were included in genome sequencing. As indicated in Table 2 the number of passages ranged from 5 to 53. No difference in plasmid content of isolates that belonged to the same MLST sequence type (ST) as Far04 (see below) was found whether they were low passage (9) or higher passage (45). The data and assembly analyses outlined below suggest that there was no loss of complete plasmids under the in vitro culture conditions chosen for this study.

Ten isolates belonging to different MLST clonal complexes (Fig. 2) and from ticks collected from different hosts, one from a razorbill, four from puffins and five from guillemots, were selected for genome sequencing (Tables 2, S2). MLST sequence types (ST) of four isolates are the same ST as Far04, ST575 (<https://pubmlst.org/borrelia/ID2798>), and one, ST804, is a single locus variant (SLV) of ST575. The other five isolates are STs ST801, ST802, ST803, ST921 and ST923 (ST921 is a SLV of ST802) (Table 2, Figs. 2 and 3). Two STs, ST801 and ST921, were unique, appearing only once in the whole dataset ($n = 388$) (Fig. 2).

Apart from the seven STs that came from our study, we downloaded *B. garinii* samples from the MLST website (www.pubmlst.org/borrelia/) available at the time representing the variety of *B. garinii* that is currently known. They included 19 North American seabird isolates from Canada, 291 Eurasian terrestrial bird and 48 human samples. The Canadian strains originate from *I. uriae* ticks collected from Atlantic puffin, black-legged kittiwake, Common Murre and razorbill in Newfoundland and Labrador (Munro et al., 2019). The terrestrial bird samples originate from various different European countries (e.g. (Norte et al., 2020; Vollmer et al., 2011)), from Russia (Mukhacheva and Kovalev, 2013), and Far Eastern Asia (Takano et al., 2011). The majority, 87%, were typed via DNA samples of *I. ricinus* and *I. persulcatus* ticks.

In a minimum spanning tree (Fig. 3) ST representing seabird *B. garinii* occur at four different locations neighboring ST251, ST743, ST244, and ST82. This positioning in the minimum spanning tree suggests several independent entries of seabird-associated *B. garinii* from terrestrial bird transmission cycles. Interestingly, ST244 has been isolated from human patients but also terrestrial birds and seabirds showing a potential overlap of these transmission cycles.

3.2. Genome types of seabird isolates

3.2.1. Genome assembly

The above 10 seabird *B. garinii* genomes were sequenced using

Table 2
Plasmid type and total length of scaffolds in kbp (without gaps) of genome elements in *Borrelia garinii* isolates from seabird ticks based on PFam32 genes.

Isolate (*in vitro passages/ tube)	PBr	20,047	Far04 [†]	17-29Z1 (22/ 5)	17-56Z1 (15/ 6)	FNG-1Z1 (9/3)	17-59N1 (45/ 3)	17-58N4 (11/ 6)	FNG-2Z14 (29/ 6)	17-54Z3 (42/7)	FNG-1Z4 (17/1)	NG-Z6 (5/ 2)	17-63N1 (53/ 3)
MLST sequence type (ST)	244	82	575	575	575	575	575	804	802	921	801	803	923
Tick host				razorbill	puffin	guillemot	puffin	puffin	guillemot	guillemot	guillemot	guillemot	Puffin
Chromosome (w/o N)	np	906,449	incomplete	899,748	899,921	899,561	899,566	899,562	901,612	900,966	902,975	900,086	903,551
cp9	np	np	np	np	np	np	np	np	np	np	np	9367	np
cp26	27,040	27,038	26,994	27,082	27,071	27,082	27,082	27,071	27,110	27,110	27,118	26,994	26,984
cp32-3	np	29,563	np	np	np	np	np	np	np	np	np	np	16,792
cp32-5	29,694												
cp32-6	np	30,422	np	np	np	np	np	np	np	np	np	np	29,682
cp32-10	34,837												
lp17**	20,872	20,863	22,433	21,950/liri	21,958/lr	21,971/ lr	21,962/lr	21,958/lir	18,468/lri	20,762/ lir	17,682/lr	20,839/l	64,069/r
lp25***	32,543	np	25,791	22,814/n	23,553/li	24,632/li	22,871/n	23,895/li	28,085/lr	28,075/r	25,058/lr	23,426/n	27,473/lr
lp28-2	np	np	np	np	np	np	np	np	np	np	np	17,359/r	np
lp28-3	17,609	26,552	np	np	np	np	np	np	26,308/l	19,876/n	31,483/ l	12,943/r	np
lp28-4	40,280	41,311	np	np	np	np	np	np	np	np	22,553/n	np	np
lp28-7	27,006	27,093	np	np	np	np	np	np	np	np	np	np	np
lp28-9****	38,262	np	27,689	18,327/l	24,775/l	23,083/l	22,724/l	23,759/l	np	np	np	np	31,477/lr
lp32-10	np	25,672	41,906	41,596/l	41,603/lri	41,687/ lr	41,608/lri	41,661/lr	26,559/l	29,026/n	np	np	*****
lp36	36,709	36,994	35,446	35,406/l	35,471/lr	35,568/ lr	35,471/lri	35,452/l	np	np	np	np	np
lp54	56,869	57,454	58,069	57,991/r	58,117/r	58,084/ lr	57,034/r	57,049/lr	55,632/l	55,700/l	57,197/lr	56,902/r	55,831/lr
Unnamed (no PFam32)										2974 (+) 3242§		3049§	37,129/l§

np = not present.

lr = left / right telomere present; liri = left / right telomere incomplete; n = no telomere sequence found (see Table S4 for further information).

* number of in vitro passages/tube.

** lp17 was obtained in two parts, one about 2500 bp, the other 19,500 bp. Compared to Far04, in all assemblies there were around 500 bp missing within a repetitive region; length may include stretches of "N".

*** lp25 assembly was fragmented in some isolates with gaps between contigs except for 17-63N1 for which PacBio sequences were available; length includes stretches of "N".

**** lp28 vls-region fragmented, size includes vls cassette fragments.

***** fused with lp17.

= partial match with lp36 and lp28-9.

+ right end of lp32-10.

§ similarity to lp36.

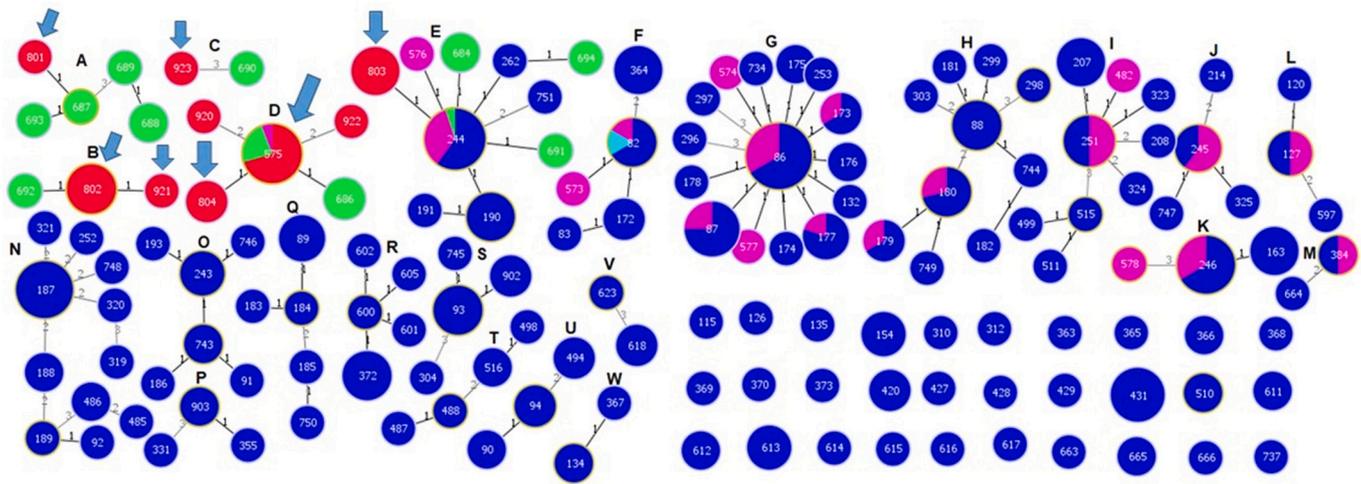


Fig. 2. GoeBURST diagram of *B. garinii* samples from terrestrial and seabird transmission cycles based on triple locus variant (TLV) which means that sequence types (STs) that differ in one, two or three alleles are being connected whilst STs that differ in four or more loci have no connection. The circles represent STs and the size of the circles is proportional to the number of samples included in the STs. Numbers on the circles represent ST numbers, numbers on the connection lines refer to allele differences. Letters A-W indicate major (≥ 3 STs) and minor (2 STs) clonal complexes. Samples included in whole genome sequencing were from clonal complex A-E and are indicated by an arrow. Sample designation are as follows: **A:** ST801: FNG-1Z4; **B:** ST802: FNG-2Z14; **C:** ST921: 17-54Z3; **D:** ST575: Far-04; **E:** ST923: 17-63N1; **F:** ST804: 17-29Z1, 17-56Z1, 17-59N1, FNG-1Z1, ST804: 17-58N4; **G:** ST803: NG-Z6. **Colour coding:** dark blue = isolate from terrestrial ticks ($n = 290$); light blue = type strain 20047; pink = human isolates ($n = 48$); green = isolate from American *I. uriae* ($n = 19$); red = isolate from European *I. uriae* ($n = 30$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Illumina MiSeq technology and one, 17-63N1, also with PacBio SMRT technology (Table 2). For most genomes, the average coverage of the chromosome was $>100\times$ (except 17-54Z3 and NG-Z6) and $>20\times$ for most plasmids, except lp25 which had a substantial lower coverage compared to the chromosome in some of the isolates (Table S3). To confirm the unusual genome type of isolate 17-63N1 (containing cp32 and a fusion plasmid, Table 2) an assembly based on long-read technology was required to allow us to confirm the plasmids.

In our isolates, the length of the chromosomes ranged from 899,562 bp to 903,551 bp. Even in samples with high coverage ($>100\times$) and a small number of chromosomal contigs ($n = 3$) was the length of the chromosome below 900 kbp. Critical assembly regions were the coding sequences for a tetratricopeptide repeat protein (corresponding to the coding region of *Imp1* in *B. burgdorferi* sensu stricto B31 (Tyler et al., 2018)) and the region encoding the rRNA loci (Fig. S1). The plasmids assembled in the 10 isolates and their comparison with plasmids of *B. garinii* isolates Far04, 20047, PBr and *B. valaisiana* VS116 are shown in Figs. S2-S10.

Plasmids cp26 and lp54 are well assembled in all isolates and are of the expected size (Table 2). In several isolates assemblies of lp17 and lp32-10 were in two contigs, likely due to repetitive regions. Such repetitive regions where the two contigs were merged start in lp17 and lp32-10 at around 19,380 bp and 998 bp, respectively. The assembly of plasmid lp25 is fragmented in all but two isolates, resulting in two to six contigs that show gaps when mapped onto lp25 of Far04 (Table S3). Whether this is the result of low coverage (at least in some isolates) is unknown. Nevertheless, the mapped sequences have a very high similarity ($\geq 99.5\%$ identity) to lp25 of Far04 (Fig. S3) suggesting that the gene content of lp25 is intact despite assembly issues in repetitive regions.

lp28-9 plasmids are present in all isolates of ST575, ST804 and ST923 (Fig. S7) and contain the variant major protein-like sequence (*vls*) locus although the silent cassette region is incompletely assembled in genomes that were generated with only Illumina reads. This locus is an important virulence determinant of *Borrelia* that allows antigen switching and immune evasion (Norris et al., 2010). lp32-10 plasmids are present in isolates 17-29Z1, 17-56Z1, FNG-1Z1, 17-59N1, 17-58N4, FNG-2Z14 and 17-54Z3. In the last two isolates lp32-10 is truncated to 26.5 and 29.0 kbp, respectively, while it is 41 kbp in the other five

isolates (Fig. S8).

Plasmid lp54, which was completely assembled in all isolates, differed in some isolates compared to Far04 in having less genes in the variable region IV of the PFam54 gene array (Fig. S11).

3.2.2. Genome structure

The genomes of five isolates, 17-29Z1, 17-56Z1, FNG-1Z1, 17-59N1 (all ST575) and 17-58N4 (ST804) are very similar and have an identical plasmid composition to Far04 (Table 2, Fig. 4, see also Figs. S2-S9 for plasmid comparisons). Four plasmids, cp26, lp17, lp25, and lp54, are present in all isolates (Table 2, Fig. 4).

The genomes of the remaining isolates are unique and some of the remarkable features of these genomes are described in more detail as follows: Isolate NG-Z6, in addition to having cp26, lp17, lp25, and lp54, possesses a cp9, an lp28-2 and an lp28-3, the latter encoding part of the *vls* locus. Its lp17 is the same as Far04 lp17 except for a 1 kbp deletion near the middle (Fig. S2). The PFam32 of lp28-2 shows 98% identity to PKo lp28-2 and the plasmid contains a sequence stretch of approximately 5 kbp that is similar to PKo lp28-2. The PFam54 gene array on lp54 has less genes (A0067, A00072, A00073, A00074) compared to the Far04 PFam54 gene array (Fig. S11).

The plasmid repertoire of FNG-1Z4 includes cp26, lp17, lp25, lp28-3 that contains the *vls* region, an lp28-4 similar to PBr, and an lp54. The genomes of FNG-2Z14 and 17-54Z3 are similar to each other in terms of plasmid numbers and structure (Table 2), except that 17-54Z3 has two unidentifiable short sequence assemblies of 2.9 kbp and 3.2 kbp that are not present in FNG-2Z14 (unidentifiable plasmids in Fig. 4). The 2.9 kbp contig matches the right end of lp32-10 of Far04 and related seabird *Borrelia* genomes (Fig. S8). The 3.2 kbp sequences is an identical match to the right end of lp36 of Far04 (not shown). Both isolates differed from Far04 in that lp28-9 is absent; an atypically short *vls* region is found in these two genomes (Fig. S5). The lp28-3 plasmid fragment in 17-54Z3, being 19,876 bp in length, is short for an lp28 plasmid suggesting that it is incomplete, a suggestion that is supported by the absence of telomeres on both sides.

The most unusual genome is that of 17-63N1: it possesses a cp26, two cp32 plasmids - cp32-3 and cp32-6, a fusion plasmid lp17 + 32-10, lp25, lp28-9, lp36 and lp54. Its lp17 is nearly 47 kbp in length and is fused to a cp32-10/lp32-10 (Fig. S2). The lp17 sequence starts from

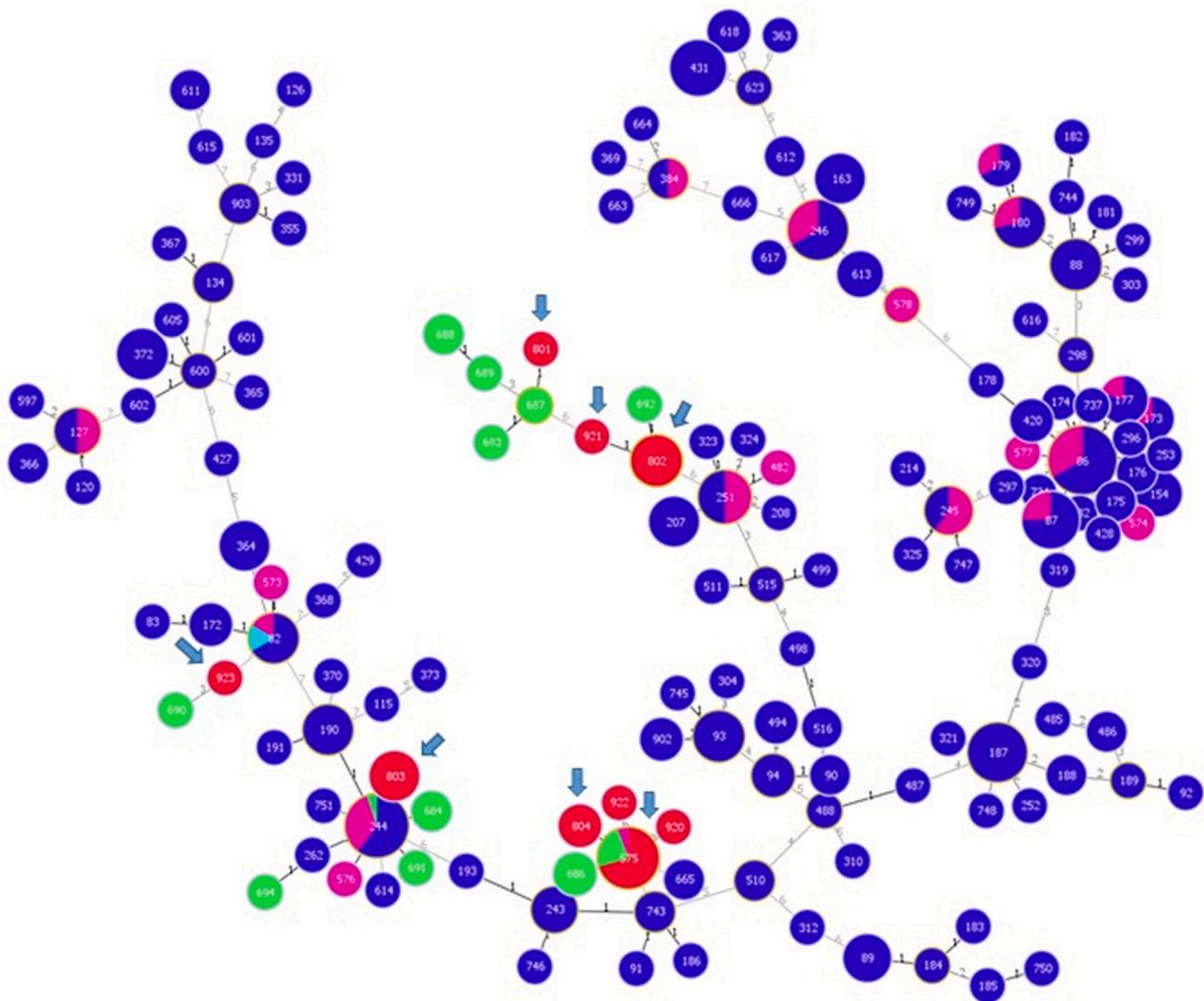


Fig. 3. Minimum spanning tree of *B. garinii* samples of terrestrial and seabird transmission cycles. Samples included in whole genome sequencing are indicated by an arrow. ST575: Far-04, 17-29Z1, 17-56Z1, 17-59N1, FNG-1Z1, ST804: 17-58N4; ST802: FNG-2Z14; ST921: 17-54Z3; ST801: FNG-1Z4; ST923: 17-63N1; ST803: NG-Z6. **Colour coding:** dark blue = isolate from terrestrial ticks (n = 290); light blue = type strain 20047; pink = human isolates (n = 48); green = isolate from American *I. uriae* (n = 19); red = isolate from European *I. uriae* (n = 30). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

about 30.5 kbp of the 5' region with a repeat region just transcriptionally upstream of the PFam32 gene that is 99% identical to lp17 PFam32 of Far04 and PBr. It has a ~ 2 kbp DNA insert compared to strains PBr and 20047 and, a 600–700 bp insert relative to Far04. The sequence left of 30.5 kbp is a cp32-like sequence. It contains an imperfect duplication of a partly deleted cp32. Its PFam32 is 98% identical to PBr cp32–10 and lp32–10 of PKo, but the plasmid does not show organizational similarity to the lp32–10 of PKo, Far04 or 20047. The lp28–9 plasmid has the *vlsE* gene and *vls* cassettes with a typical inverted repeat between them (Fig. S7). An unidentified plasmid (PFam32 missing) of approximately 37 kbp has 14–15 kbp similarity to lp36 of 20047 and Far04.

3.3. Phylogenetic analyses of surface proteins potentially linked to host or vector associations

We did not find any obvious association of the different genome types with marine avian hosts. Ticks harboring *B. garinii* of ST575/ST804 have the same genome type as Far04 and were collected from razorbills, puffins, or guillemots. The unique genome types were also isolated from *I. uriae* collected from guillemots and the isolate with the

unusual genome, 17-63N1, was from a tick collected from a puffin. We used phylogenetic analyses to investigate whether genes that had previously been linked to host or vector association would give a cue towards such associations. Included sequences were the PFam54 gene array containing complement-regulatory acquiring surface proteins (CRASPs) (Wywiał et al., 2009), *ospA* (Pal et al., 2004) and *ospC* (Fingerle et al., 2007; Tilly et al., 2007) (Figs. S12 and S13) (see methods for details and Table S5 for samples included in these analyses).

Previous research divided the PFam54 into five regions (I, II, III, IV, V), with region IV being diverse (Wywiał et al., 2009). All isolates possess homologues to A0063, A0064, A0066, A0068, A0071, and A0075 of Far04. Some differences are present in region IV of the PFam54 gene array compared to Far04, but these differences did not match the bird host species (Fig. S11). Several of the genes present in Far04 are missing in our isolates, e.g. there are no homologues to BgFar04-A0065 and -A0070. In FNG-1Z4 the loci encoding BgFar04-A0068 and -A0073 are duplicated. Genes that cluster as sister to BgFar04-A0070 are found in FNG-1Z4, 17-54Z3 and FNG-2Z14. The PFam54 gene arrays of two isolates 17-54Z3 and FNG-2Z14 are identical, both having a duplication of homologues of BgFar04-A0066 and no homologue sequences for

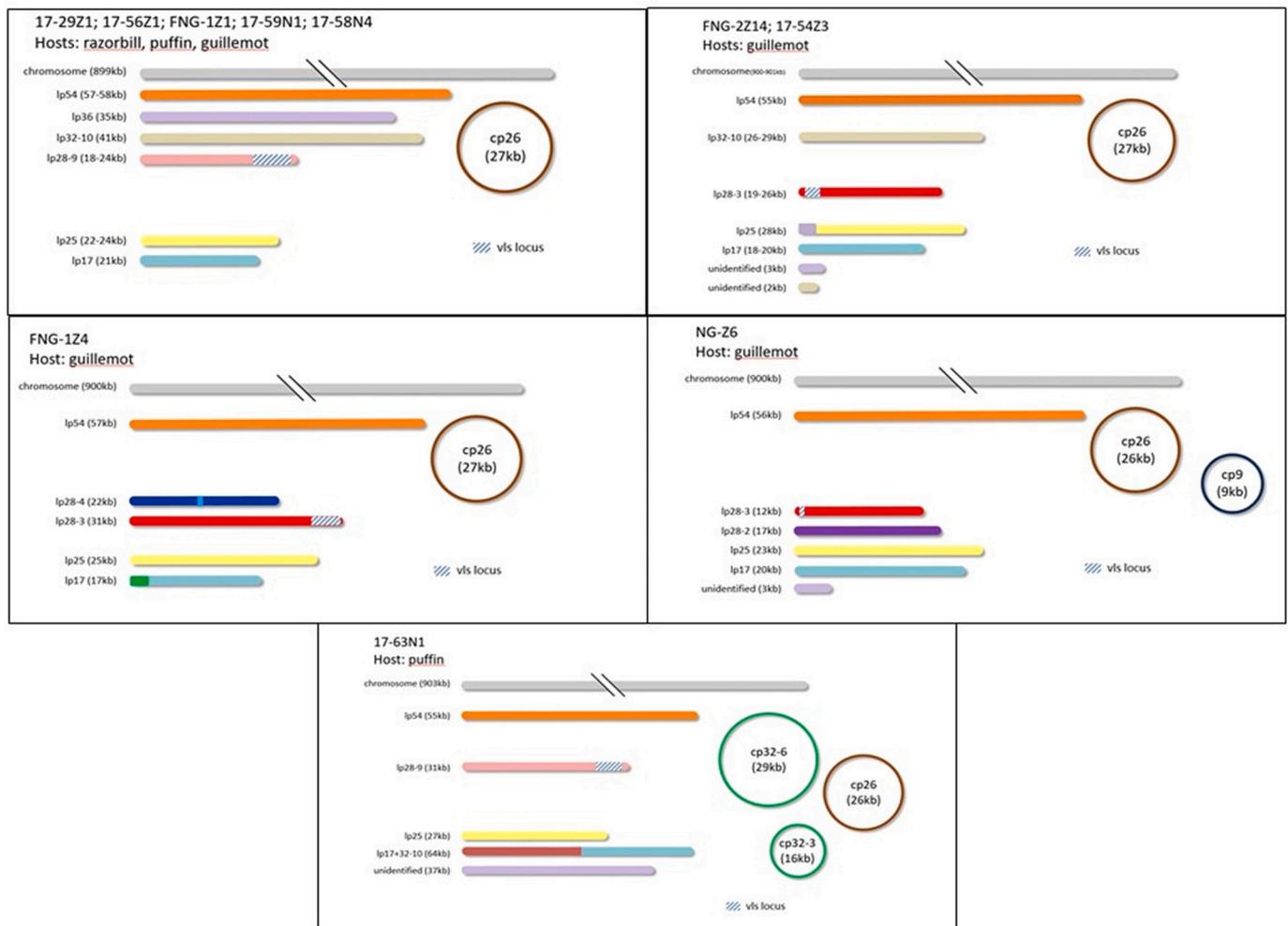


Fig. 4. Genome types in *B. garinii*. The schematic drawing shows genome types which were defined by presence of plasmids. Plasmids containing the *vls*-locus, one of the major virulence determinants, are indicated. ST575/ST804 having been isolated from ticks collected from razorbill, puffin and guillemot have identical genome types. FNG-2Z14 and 17-54Z3 isolated from ticks collected from guillemot have also very similar genome structure. Genome types of FNG-1Z4 (*I. uriae* from guillemot), NG-Z6 (*I. uriae* from guillemot) and 17-63N1 (*I. uriae* from puffin) are unique. Importantly, 17-63N1 carries two cp32 plasmids that were previously not found in seabird associated *B. garinii* but are present in *B. garinii* type strain 20047 (see Table 2). Unidentifiable plasmids have no PFam32.

A0069, A0072 and A0074 (Fig. S11). The isolates belonging to ST575/ST804 have a homologous sequence to CRASPs of the PFam54 gene array on lp25 (except 17-56Z1) and on lp32–10. Homologous sequences are present on the corresponding plasmids in Far04 and 20047, but in the latter both loci were on lp32–10.

Isolates belonging to ST575 and ST804 have the same *ospA*-type as Far04 while the sequence of 17-63N1 forms a sister clade (Fig. S13). NG-Z6 had an *ospA*-type 3 and FNG-1Z4 clusters close to it. 17-54Z3 and FNG-2Z14 do not cluster with any other *B. garinii* *ospA*-type but form their own terminal clade. A similar picture emerged for *ospC* – all isolates belonging to ST575, ST804 and NG-Z6 have an *ospC*-type identical to Far04 (Fig. S13). 17-54Z3 and FNG-2Z14 have identical *ospC* and *ospA*-types underlining the close relationship of these two isolates. The *ospC* of FNG-1Z4 is similar to PLa (ST245) whilst the *ospC* of 17-63N1 is unique. Both molecules show no clustering relative to avian host species.

4. Discussion

In this study we aimed to obtain additional isolates of *Borrelia* species from seabird - *I. uriae* transmission cycles to test the hypothesis that the small genome found in Far04 reflects a niche adaptation. Although in PCR analyses other species, e.g. *B. burgdorferi* sensu stricto, were identified in the collected *I. uriae* ticks, none of the cultures became positive

for species other than *B. garinii*. For *B. garinii*, 17 samples were adapted to in vitro culture. Whole genomes were sequenced for ten of them.

4.1. Ecology of the seabird transmission cycle

The *B. garinii* investigated here were from *I. uriae* ticks collected in 2017 and 2018 for the purpose of this study from seabirds breeding sympatrically within a single, large breeding colony on the island Hornøya in northern Norway. Sample collection included ticks collected directly from the birds or nests (2017) of four different seabird species, namely the common guillemot, black-legged kittiwake, Atlantic puffin and razorbill. Ticks were also included that were found off-host (2018), but given the ecology of the seabirds, these ticks are likely from puffin hosts. Indeed, past studies on this island have demonstrated the presence of distinct tick populations (ie, host races) infesting the different seabird host species (e.g., (Dietrich et al., 2014; McCoy et al., 2001)). The probability of humans encountering each race depends on the breeding ecology of the host species, with puffin ticks being more active on grassy slopes where puffins breed than ticks of the other three seabird species which are largely confined to particular breeding zones on the cliff face (McCoy et al., 2003). Interestingly, at the colony level, some differences in *Borrelia* infection dynamics have been demonstrated among the different tick races, with puffin ticks having a higher prevalence and

higher intensities of infection than the other tick host races (Gomez-Diaz et al., 2010). However, *Borrelia* strains were not found to be genetically structured among these host-associated populations, and it was concluded that enough sharing of tick bites may occur to transmit *Borrelia* infections among different seabird species (Gomez-Diaz et al., 2011). However, to date, no indepth analysis of marine *Borrelia* genomes has been performed and we still know little about its interaction with terrestrial transmission cycles.

4.2. Completeness of the sequenced genomes

The isolates were sequenced using Illumina MiSeq technology which provides short reads in high abundance, often covering the whole genome. Illumina sequencing is a very attractive technology as it provides fast and accurate reads for genomes at low cost per single genome (Quail et al., 2012). However, a limitation is that repetitive regions of the genome may prevent the assembly of chromosomes or plasmids into a single contig (Margos et al., 2017; Tyler et al., 2018). Accordingly, we found that the chromosomes were usually divided into three or more contigs; gaps appearing in known repetitive regions of the chromosome such as *lmp1* (around 230,000 bp from the left end of the chromosome of B31). In addition, lp17 was usually not completely assembled due to a repetitive region at positions 2330–3012 (19,425–20,104 in a reverse complement representation of lp17) which was found in lp17 of Far04 when searching for tandem repeats using the website (<http://tandem.bu.edu/trf/>). Longer reads will be necessary to fully assemble these *Borrelia* plasmids but the here assembled genomes provided ample information on the genomic features of seabird-associated *B. garinii*.

4.3. Genomic features of seabird-associated *Borrelia garinii*

Before our study, the genome of only a single isolate (Far04) of *B. garinii* from a seabird host (Atlantic puffin) sampled in the Faroe Islands had been sequenced (Gylfe et al., 1999). The genome turned out to be rather small containing seven plasmid, one circular (cp26) and six linear plasmids (Casjens et al., 2011). The most distinguishing feature from completely sequenced genomes of *B. garinii* from terrestrial birds was the absence of cp32 plasmids (Brisson et al., 2013). One of the questions addressed by the latter study was whether the absence of cp32 plasmids was due to in vitro cultivation of isolate Far04 accompanied by plasmid loss or whether cp32 absence also occurs in nature (Casjens et al., 2011; Brisson et al., 2013). As the majority of genomes of seabird-associated *B. garinii* isolated in the present study (except 17-63N1) lacked cp32, we suggest that this is a natural phenomenon, not an artifact of in vitro cultivation. Plasmids of the cp32 family represent prophages. Apart from this, they encode an interesting protein family, the *erp* (OspE/F-related protein) family. The genes (that have also been termed *ospE*, *ospF* or *elp*), encode outer surface proteins that bind to laminin, plasminogen, the complement regulator factor H and seem to be important in host- or vector-interaction (Brisson et al., 2013; Stevenson et al., 2000). It has been suggested that these proteins may enable Lyme spirochetes to infect a wide range of vertebrate hosts (Stevenson et al., 2002). Accordingly, in our study, we aimed to obtain more seabird-associated isolates for further exploration of the genome structure of strains circulating in marine ecosystems and to investigate whether its genome structure may be an ecological niche adaptation. However, isolates lacking cp32 plasmids were isolated from ticks feeding on the different hosts and further experimental evidence would be required to confirm whether the different host species are equally reservoir competent and whether these isolates would endure a terrestrial transmission cycle.

A remarkable feature found during our analysis was that the genomes of the ten isolates fall into five genomic categories differing in their plasmid content. Notably, this genome structure was in accordance with the MLST types: all isolates belonging to ST575 and ST804 (the latter is an SLV of ST575 which means that they are closely related) have

the same plasmid content as Far04, i.e. one circular plasmid, cp26, and six linear plasmids including lp17, lp25, lp28–9, lp32–10, lp36 and lp54. The two isolates FNG-2Z14 and 17-54Z3 representing ST802 and ST921 (SLVs of each other) have very similar plasmids content and structure, except that 17-54Z3 possesses two unidentified plasmid fragments that were not found in the FNG-2Z14 assembly. Both genomes contain a fragment of lp28–3 encoding the *vls* locus. The three remaining isolates have unique genome structures: FNG-1Z4 possesses an lp28–3 and an lp28–4, but does not have lp28–9, lp32–10 or lp36; NG-Z6 has an lp28–2 and an lp28–3, but lacks lp28–9, lp32–10 and a complete lp36 (only a 3 kbp fragment that matches the right end of lp36 of Far04 was found). Isolate 17-63N1 differs from the other genome types: it lacks lp32–10, but possesses three cp32 plasmids, one of which is fused to lp17. Loss or gain of genetic material is a common feature in niche adaptation of pathogens and bacterial pathogens often carry adaptive loci on plasmids (de Been et al., 2014; Hinnebusch, 1997, 2003). In *Borrelia*, isolates belonging to the generalist species *B. burgdorferi* s.s. carry from 12 to 21 plasmids (Casjens et al., 2017), whilst in other species of *Borrelia*, the number of plasmids can vary from four to 17 plasmids (Casjens et al., 2018). Among the *B. garinii* samples sequenced to date, the marine isolate Far04 was found to harbour seven plasmids, the lowest number found in this species; other fully sequenced *B. garinii* isolates, 20047 and PBr possess 11 plasmids, and CIP 103362 has 12 (Casjens et al., 2018; Margos et al., 2018). As mentioned above, Far04 did not contain any cp32 plasmids, while the two *B. garinii* isolates associated with terrestrial transmission cycles (20047 and PBr) did contain cp32 plasmids. In our study we found one (17-63N1) out of the ten sequenced genomes had three cp32 plasmids, whereas these plasmids were entirely absent from the others. We suggest that the low number and plasmid variation we found in *B. garinii* may correspond to a niche adaptation to the marine transmission cycle.

Plasmid types can be identified through BLAST searches using PFam32 sequences (Casjens et al., 2012), even in genomes that are not assembled to completeness (Margos et al., 2019; Margos et al., 2017). In our study, not all plasmids were assembled to completeness and, for some plasmids such as lp17 or lp25, fragmented contigs were obtained due to presence of repetitive sequences (lp17) or for unknown reasons (lp25). Nevertheless, several of the samples showed the same number and types of plasmids as Far04. This and the differences noticed in the genome of 17-63N1 (i.e. the presence of three cp32 plasmids) begs the question of whether the loss of cp32 plasmids may be an adaptation to the seabird transmission cycle which may constitute a narrower niche. There are different genomic subgroups within our collection of genome sequences, but the different subgroups did not appear to be associated with the different host bird species investigated. It may, nevertheless, suggest that they have different niches: one of the subgroups might be better adapted to multiple bird host species, while another might have a narrower range of hosts. It would be interesting to (i) sequence further genomes of seabird isolates of *B. garinii* and (ii) to conduct in vitro studies to verify whether seabird associated *B. garinii* isolates are able to resist complement active serum of the four different marine bird species or passerine birds involved in terrestrial transmission cycles and (iii) whether they can readily be transmitted by ticks other than *I. uriae*.

We currently have no explanation for the low coverage of plasmid lp25 in our genomes. While slight variations in the coverage of different genomic elements appear to be normal (Margos et al., 2017), coverage of lp25 was up to 10× less than for other plasmids. This may be an artifact of the isolate and in vitro culturing (Grimm et al., 2003) or it may be that not every bacterial cell in the population carries an lp25. Single cell sequencing may help to reveal whether this is the case or not. Another explanation might be that lp25 is particularly difficult for library preparation for Illumina sequencing. Low coverage of this plasmid may also be the reason for the poor assembly success of this plasmid as this is often associated with poor assembly of genomes.

Does the genome structure of seabird B. garinii strains reflect its history?

The minimum spanning tree (Fig. 3) indicates the relationship

between *B. garinii* from terrestrial and seabird transmission cycles and suggests several independent introduction events. The tree shows that 17-63N1 (ST923) is neighbor to ST82, the ST of 20047. The genome of 20047 also contains cp32-3 and cp32-6, as has 17-63N1. ST803 (NG-Z6) neighbors ST244, the ST of PBr. As PBr, NG-Z6 has an lp28-3 plasmid containing the *vls* locus and its *ospA* is of the same type as PBr. 17-54Z3 (ST921) and FNG-2Z14 (ST802) neighbor ST251 which is the ST of PKuf and PBes, both are isolates from human patients (see www.pubmlst.org/borrelia/). Interestingly, the genome of PBes carries a cp9 plasmid which was not found in ST802 or ST921, but was in NG-Z6. Thus, it seems that while the genome type may have some elements that reflect strain history, others may have been lost or acquired via horizontal gene transfer.

5. Conclusion

In conclusion, we found a high degree of genomic diversity in *Borrelia*-isolates obtained from the avian seabird – *I. uriae* transmission cycle. Five different genome types are present among the 10 sequenced genomes coming from a seabird colony on the island of Hornøya. There was no apparent association between genome type, *ospA*-, *ospC*-, or CRASP-types with avian host species. Why this diversity exists and how it is maintained is one of the big questions in *Borrelia* research and warrants further investigation.

Authors contributions

GM, KDM, VF – conceptualization; MD, MH, SH, CH, SC, SH, MS – collection of materials, formal analysis of data, data curation; GM, KDM, VF, SC – original draft; GM, KDM, VF, SC, AS – review and editing; all authors have approved the manuscript.

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Ethical approval

No Ethical approval was required for the work.

Consent to publish

Consent to publish is not required as no personal details are being revealed.

Credit Author Statement.

GM, KDM, VF – conceptualization; MD, MH, SH, CH, SC, SH, MS – collection of materials, formal analysis of data, data curation; GM, KDM, VF, SC – original draft; GM, KDM, VF, SC, AS – review and editing; all authors have approved the manuscript.

Data statement

Assembled genomes and sequence reads are available in GenBank via the following accession numbers under BioProject PRJNA327303: CP119374-CP119381 (SAMN32860459, 17-29Z1); CP118290-CP118297 genome, OQ450372, OQ450373 part of *vls* locus (SAMN32860460, 17-56Z1); CP119358-CP119365 genome, OQ450374, OQ450375 part of *vls* locus (SAMN32860461, FNG-1Z1); CP118298-CP118305 genome, OQ450376, OQ450377 part of *vls* locus (SAMN32860462, 17-58N4); CP119383-CP119390 genome, OQ450378 part of *vls* locus (SAMN32860463, 17-59N1); CP117887-CP117893

(SAMN32860464, FNG-2Z14); CP117894-CP117901 (SAMN32860465, 17-54Z3); CP117902-CP117908 (SAMN32860466, FNG-1Z4); CP117909-CP117917 (SAMN32860467, NG-Z6); CP117839-CP117847 (SAMN32949868, 17-63N1).

Cultures are available on request from the National Reference Center for *Borrelia*, Veterinärstr. 2, 85764 Oberschleissheim, Germany. Email: Volker.fingerle@igl.bayern.de

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Karen McCoy reports financial support was provided by French National Research Agency. Karen McCoy reports financial support was provided by French Polar Institute Paul Emile Victor. Volker Fingerle reports financial support was provided by Robert Koch Institute. Volker Fingerle reports a relationship with Pfizer that includes: consulting or advisory and travel reimbursement. co-author advisory and consultative activities - V.F. for QCMD, INSTANT, ESGBOR, Global Lyme Alliance.

Data availability

Sequence data have been submitted to NCBI GenBank

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meeqid.2023.105502>.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9, 75. <https://doi.org/10.1186/1471-2164-9-75>. PMID: 18261238; PMCID: PMC2265698.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comp. Biol.* 19, 455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Becker, N.S., Margos, G., Blum, H., Krebs, S., Graf, A., Lane, R.S., Castillo-Ramirez, S., Sing, A., Fingerle, V., 2016. Recurrent evolution of host and vector association in bacteria of the *Borrelia burgdorferi* sensu lato species complex. *BMC Genomics* 17, 734. <https://doi.org/10.1186/s12864-016-3016-4>.
- Becker, N.S., Rollins, R.E., Nosenko, K., Paulus, A., Martin, S., Krebs, S., Takano, A., Sato, K., Kovalev, S.Y., Kawabata, H., Fingerle, V., Margos, G., 2020. High conservation combined with high plasticity: genomics and evolution of *Borrelia bavariensis*. *BMC Genomics* 21, 702. <https://doi.org/10.1186/s12864-020-07054-3>.

- Bosi, E., Donati, B., Galardini, M., Brunetti, S., Sagot, M.F., Lio, P., Crescenzi, P., Fani, R., Fondi, M., 2015. MeDuSa: a multi-draft based scaffold. *Bioinformatics* 31, 2443–2451. <https://doi.org/10.1093/bioinformatics/btv171>.
- Brisson, D., Zhou, W., Jutras, B.L., Casjens, S., Stevenson, B., 2013. Distribution of cp32 prophages among Lyme disease-causing spirochetes and natural diversity of their lipoprotein-encoding *erp* loci. *Appl. Environ. Microbiol.* 79, 4115–4128. <https://doi.org/10.1128/AEM.00817-13>.
- Casjens, S., Mongodin, E., Qiu, W.-G., Dunn, J., Luft, B., Fraser-Liggett, C., Schutzer, S., 2011. Whole-Genome Sequences of Two *Borrelia afzelii* and Two *Borrelia garinii* Lyme Disease Agent Isolates. *J. Bacteriol.* 193, 6995–6996. <https://doi.org/10.1128/JB.05951-11>.
- Casjens, S.R., Mongodin, E.F., Qiu, W.G., Luft, B.J., Schutzer, S.E., Gilcrease, E.B., Huang, W.M., Vujanovic, 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>.
- Casjens, S.R., Gilcrease, E.B., Vujanovic, M., Mongodin, E.F., Luft, B.J., Schutzer, S.E., Fraser, C.M., Qiu, W.G., 2017. Plasmid diversity and phylogenetic consistency in the Lyme disease agent *Borrelia burgdorferi*. *BMC Genomics* 18, 165. <https://doi.org/10.1186/s12864-017-3553-5>.
- Casjens, S.R., Di, L., Akther, S., Mongodin, E.F., Luft, B.J., Schutzer, S.E., Fraser, C.M., Qiu, W.G., 2018. Primordial origin and diversification of plasmids in Lyme disease agent bacteria. *BMC Genomics* 19, 218. <https://doi.org/10.1186/s12864-018-4597-x>.
- Comstedt, P., Bergstrom, S., Olsen, B., Garpmo, U., Marjavaara, L., Mejlon, H., Barbour, A.G., Bunikis, J., 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. *Emerg. Infect. Dis.* 12, 1087–1095.
- Comstedt, P., Asokliene, L., Eliasson, I., Olsen, B., Wallenstein, A., Bunikis, J., Bergstrom, S., 2009. Complex population structure of Lyme borreliosis group spirochete *Borrelia garinii* in subarctic Eurasia. *PLoS One* 4, e5841.
- Comstedt, P., Jakobsson, T., Bergstrom, S., 2011. Global ecology and epidemiology of *Borrelia garinii* spirochetes. *Infect. Ecol. Epidemiol.* 1 <https://doi.org/10.3402/iee.v1i0.9545>.
- Darling, A.C., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* 14, 1394–1403. <https://doi.org/10.1101/gr.2289704>.
- de Been, M., Lanza, V.F., de Toro, M., Scharring, J., Dohmen, W., Du, Y., Hu, J., Lei, Y., Li, N., Tooming-Klunderud, A., Heederik, D.J., Fluit, A.C., Bonten, M.J., Willems, R., de la Cruz, F., van Schaik, W., 2014. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. *PLoS Genet.* 10, e1004776 <https://doi.org/10.1371/journal.pgen.1004776>.
- Dietrich, M., Lobato, E., Bouludier, T., McCoy, K.D., 2014. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J. Anim. Ecol.* 83, 576–587. <https://doi.org/10.1111/1365-2656.12170>.
- Dubská, L., Literák, I., Kocianová, E., Taragelova, V., Sychra, O., 2009. Differential role of passerine birds in distribution of *Borrelia* spirochetes, based on data from ticks collected from birds during the postbreeding migration period in Central Europe. *Appl. Environ. Microbiol.* 75, 596–602.
- Duneau, D., Bouludier, 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.
- Eisen, L., 2020. Vector competence studies with hard ticks and *Borrelia burgdorferi* sensu lato spirochetes: a review. *Ticks. Tick. Borne. Dis.* 11, 101359. <https://doi.org/10.1016/j.ttbdis.2019.101359>.
- Fingerle, V., Goettner, G., Gern, L., Wilske, B., Schulte-Spechtel, U., 2007. Complementation of a *Borrelia afzelii* OspC mutant highlights the crucial role of OspC for dissemination of *Borrelia afzelii* in *Ixodes ricinus*. *Int. J. Med. Microbiol.* 297, 97–107.
- Fingerle, V., Schulte-Spechtel, U.C., Ruzic-Sabljic, E., Leonhard, S., Hofmann, H., Weber, K., Pfister, K., Strle, F., Wilske, B., 2008. Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. *Int. J. Med. Microbiol.* 298, 279–290.
- Francisco, A.P., Bugalho, M., Ramirez, M., Carrico, J.A., 2009. Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. *BMC Bioinform.* 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>.
- 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>.
- Gomez-Diaz, E., Bouludier, 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>.
- Grimm, D., Elias, A.F., Tilly, K., Rosa, P.A., 2003. Plasmid stability during in vitro propagation of *Borrelia burgdorferi* assessed at a clonal level. *Infect. Immun.* 71, 3138–3145.
- Grimm, D., Tilly, K., Byram, R., Stewart, P.E., Krum, J.G., Bueschel, D.M., Schwan, T.G., Policastro, P.F., Elias, A.F., Rosa, P.A., 2004. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3142–3147.
- Gylfe, Olsen, B., Strasevicius, D., Marti Ras, N., Weihe, P., Noppa, L., Ostberg, Y., Baranton, G., Bergstrom, 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.
- Hart, T., Nguyen, N.T.T., Nowak, N.A., Zhang, F., Linhardt, R.J., Diuk-Wasser, M., Ram, S., Kraiczky, P., Lin, Y.P., 2018. Polymorphic factor H-binding activity of CspA protects Lyme borreliae from the host complement in feeding ticks to facilitate tick-to-host transmission. *PLoS Pathog.* 14, e1007106 <https://doi.org/10.1371/journal.ppat.1007106>.
- Hart, T.M., Dupuis 2nd, A.P., Tufts, D.M., Blom, A.M., Starkey, S.R., Rego, R.O.M., Ram, S., Kraiczky, P., Kramer, L.D., Diuk-Wasser, M.A., Kolokotronis, S.O., Lin, Y.P., 2021. Host tropism determination by convergent evolution of immunological evasion in the Lyme disease system. *PLoS Pathog.* 17, e1009801 <https://doi.org/10.1371/journal.ppat.1009801>.
- Hepner, S., Fingerle, V., Duscher, G.G., Felsberger, G., Marosevic, D., Rollins, R.E., Okeyo, M., Sing, A., Margos, G., 2020. Population structure of *Borrelia turdica* from Greece and Turkey. *Infect. Genet. Evol.* 77, 104050. <https://doi.org/10.1016/j.meegid.2019.104050>.
- Hepner, S., Kuleshov, K., Tooming-Kunderud, A., Alig, N., Gofton, A., Casjens, S., Rollins, R.E., Dangel, A., Mourkas, E., Sheppard, S.K., Wieser, A., Hubner, J., Sing, A., Fingerle, V., Margos, G., 2023. A high fidelity approach to assembling the complex *Borrelia* genome. *BMC Genomics* 24, 401. <https://doi.org/10.1186/s12864-023-09500-4>.
- Heylen, D., Tijssie, E., Fonville, M., Matthysen, E., Sprong, H., 2013. Transmission dynamics of *Borrelia burgdorferi* s.l. in a bird tick community. *Environ. Microbiol.* 15, 663–673. <https://doi.org/10.1111/1462-2920.12059>.
- Heylen, D., Matthysen, E., Fonville, M., Sprong, H., 2014. Songbirds as general transmitters but selective amplifiers of *Borrelia burgdorferi* sensu lato genotypes in *Ixodes ricinus* ticks. *Environ. Microbiol.* 16, 2859–2868. <https://doi.org/10.1111/1462-2920.12304>.
- Hinnebusch, B.J., 1997. Bubonic plague: a molecular genetic case history of the emergence of an infectious disease. *J. Mol. Med. (Berl)* 75, 645–652.
- Hinnebusch, B.J., 2003. Transmission factors: *Yersinia pestis* genes required to infect the flea vector of plague. *Adv. Exp. Med. Biol.* 529, 55–62. https://doi.org/10.1007/0-306-48416-1_11.
- Jacquot, M., Gonnet, M., Ferquel, E., Abrial, D., Claude, A., Gasqui, P., Choumet, V., Charras-Garrido, M., Garnier, M., Faure, B., Sertour, N., Dorr, N., De Goer, J., Vourc'h, G., Bailly, X., 2014. Comparative population genomics of the *Borrelia burgdorferi* species complex reveals high degree of genetic isolation among species and underscores benefits and constraints to studying intra-specific epidemiological processes. *PLoS One* 9, e94384. <https://doi.org/10.1371/journal.pone.0094384>.
- Korenberg, E., Kovalevskii, Y.V., Kryuchevnikov, V.N., Gorelova, N.B., 1991. Tick *Ixodes persulcatus* Schulze 1930 as a vector of *Borrelia burgdorferi*. In: Dusbabek, F., Bukva, V. (Eds.), *Modern Acarology*. Academia, Prague, pp. 119–123.
- Korenberg, E.I., Gorelova, N.B., Kovalevskii, Y.V., 2002. Ecology of *Borrelia burgdorferi* sensu lato in Russia. In: Gray, J., Kahl, O., Lane, R.S., Stanek, G. (Eds.), *Lyme Borreliosis: Biology, Epidemiology and Control*. CABI Publishing, Wallingford.
- Korenberg, E.I., Nefedova, V.V., Romanenko, V.N., Gorelova, N.B., 2010. The tick *Ixodes pavlovskyi* as a host of spirochetes pathogenic for humans and its possible role in the epizootiology and epidemiology of borrelioses. *Vector. Borne. Zoonotic Dis.* 10, 453–458. <https://doi.org/10.1089/vbz.2009.0033>.
- Kraiczky, P., Stevenson, B., 2013. Complement regulator-acquiring surface proteins of *Borrelia burgdorferi*: structure, function and regulation of gene expression. *Ticks. Tick. Borne. Dis.* 4, 26–34. <https://doi.org/10.1016/j.ttbdis.2012.10.039>.
- Kurtenbach, K., Hanincova, 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.
- Lin, Y.P., Frye, A.M., Nowak, T.A., Kraiczky, P., 2020. New insights into CRASP-mediated complement evasion in the Lyme disease zoonotic cycle. *Front. Cell. Infect. Microbiol.* 10, 1. <https://doi.org/10.3389/fcimb.2020.00001>.
- 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.
- Margos, G., Hepner, S., Mang, C., Marosevic, D., Reynolds, S.E., Krebs, S., Sing, A., Derdakova, M., Reiter, M.A., Fingerle, V., 2017. Lost in plasmids: next generation sequencing and the complex genome of the tick-borne pathogen *Borrelia burgdorferi*. *BMC Genomics* 18, 422. <https://doi.org/10.1186/s12864-017-3804-5>.
- Margos, G., Gofton, A., Wibberg, D., Dangel, A., Marosevic, D., Loh, S.M., Oskam, C., Fingerle, V., 2018. The genus *Borrelia* reloaded. *PLoS One* 13, e0208432. <https://doi.org/10.1371/journal.pone.0208432>.
- Margos, G., Becker, N.S., Fingerle, V., Sing, A., Ramos, J.A., Carvalho, I.L., Norte, A.C., 2019. Core genome phylogenetic analysis of the avian associated *Borrelia turdica* indicates a close relationship to *Borrelia garinii*. *Mol. Phylogenet. Evol.* 131, 93–98. <https://doi.org/10.1016/j.ympev.2018.10.044>.
- Masuzawa, T., 2004. Terrestrial distribution of the Lyme borreliosis agent *Borrelia burgdorferi* sensu lato in East Asia. *Jpn. J. Infect. Dis.* 57, 229–235.
- McCoy, K.D., Bouludier, T., Tirard, C., Michalakakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J. Evol. Biol.* 14, 395–405.
- McCoy, K.D., Bouludier, T., Tirard, C., Michalakakis, Y., 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution* 57, 288–296.
- McCoy, K.D., Toty, C., Dupraz, M., Tornos, J., Gamble, A., Garnier, R., Descamps, S., Bouludier, T., 2022. Climate change in the Arctic: testing the poleward expansion of

- ticks and tick-borne diseases. bioRxiv 2022.07.20.500759. <https://doi.org/10.1101/2022.07.20.500759>.
- Mediannikov, O.Y., Ivanov, L., Zdanovskaya, N., Vorobyova, R., Sidelnikov, Y., Fournier, P.E., Tarasevich, I., Raoult, D., 2005. Diversity of *Borrelia burgdorferi* sensu lato in Russian Far East. *Microbiol. Immunol.* 49, 191–197. <https://doi.org/10.1111/j.1348-0421.2005.tb03717.x>.
- Muhleip, J.J., Lin, Y.P., Kraiczky, P., 2018. Further insights into the interaction of human and animal complement regulator factor H with viable Lyme disease spirochetes. *Front. Vet. Sci.* 5, 346. <https://doi.org/10.3389/fvets.2018.00346>.
- Mukhacheva, T.A., Kovalev, S.Y., 2013. Multilocus sequence analysis of *Borrelia burgdorferi* s.l. in Russia. *Ticks. Tick. Borne. Dis.* 4, 275–279.
- Munro, H.J., Ogden, N.H., Mechai, S., Lindsay, L.R., Robertson, G.J., Whitney, H., Lang, A.S., 2019. Genetic diversity of *Borrelia garinii* from *Ixodes uriae* collected in seabird colonies of the northwestern Atlantic Ocean. *Ticks. Tick. Borne. Dis.* 10, 101255. <https://doi.org/10.1016/j.ttbdis.2019.06.014>.
- Norris, S.J., Coburn, J., Leong, J.M., Hu, L.T., Höök, M., 2010. Pathobiology of Lyme disease *Borrelia*. In: Samuels, D.S., Radolf, J.D. (Eds.), *Borrelia - Molecular Biology, Host Interaction and Pathogenesis*. Caister Academic Press, Norfolk, UK, pp. 299–332.
- Norte, A.C., Ramos, J.A., Gern, L., Nuncio, M.S., Lopes de Carvalho, I., 2013. Birds as reservoirs for *Borrelia burgdorferi* s.l. in Western Europe: circulation of *B. turdi* and other genospecies in bird-tick cycles in Portugal. *Environ. Microbiol.* 15, 386–397. <https://doi.org/10.1111/j.1462-2920.2012.02834.x>.
- Norte, A.C., Margos, G., Becker, N.S., Albino Ramos, J., Nuncio, M.S., Fingerle, V., Araujo, P.M., Adamik, P., Alivizatos, H., Barba, E., Barrientos, R., Cauchard, L., Csorgo, T., Diakou, A., Dingemans, N.J., Doligez, B., Dubiec, A., Eeva, T., Flaisz, B., Grim, T., Hau, M., Heylen, D., Hornok, S., Kazantzidis, S., Kovats, D., Krause, F., Literak, I., Mand, R., Mentessana, L., Morinay, J., Mutanen, M., Neto, J.M., Novakova, M., Sanz, J.J., Pascoal da Silva, L., Sprong, H., Tirri, I.S., Torok, J., Trilar, T., Tyller, Z., Visser, M.E., Lopes de Carvalho, I., 2020. Host dispersal shapes the population structure of a tick-borne bacterial pathogen. *Mol. Ecol.* 29, 485–501. <https://doi.org/10.1111/mec.15336>.
- Olsen, B., Jaenson, T.G., Noppa, L., Bunikis, J., Bergstrom, S., 1993. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. *Nature* 362, 340–342.
- Olsen, B., Duffy, D.C., Jaenson, T.G., Gylfe, A., Bonnedahl, J., Bergstrom, S., 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. *J. Clin. Microbiol.* 33, 3270–3274.
- Pal, U., Li, X., Wang, T., Montgomery, R.R., Ramamoorthi, N., Desilva, A.M., Bao, F., Yang, X., Pypaert, M., Pradhan, D., Kantor, F.S., Telford, S., Anderson, J.F., Fikrig, E., 2004. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. *Cell* 119, 457–468. <https://doi.org/10.1016/j.cell.2004.10.027>.
- Patton, T.G., Brandt, K.S., Nolder, C., Clifton, D.R., Carroll, J.A., Gilmore, R.D., 2013. *Borrelia burgdorferi* bba66 gene inactivation results in attenuated mouse infection by tick transmission. *Infect. Immun.* 81, 2488–2498. <https://doi.org/10.1128/IAI.00140-13>.
- Preac-Mursic, V., Wilske, B., Schierz, G., 1986. European *Borrelia burgdorferi* isolated from humans and ticks culture conditions and antibiotic susceptibility. *Zentralbl. Bakteriologie Mikrobiol. Hyg. A.* 263, 112–118.
- Quail, M.A., Smith, M., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R., Bertoni, A., Swerdlow, H.P., Gu, Y., 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* 13, 341. <https://doi.org/10.1186/1471-2164-13-341>. PMID: 22827831; PMCID: PMC3431227.
- Rollins, R.E., Sato, K., Nakao, M., Tawfeeq, M.T., Herrera-Mesias, F., Pereira, R.J., Kovalev, S., Margos, G., Fingerle, V., Kawabata, H., Becker, N.S., 2022. Out of Asia? Expansion of Eurasian Lyme borreliosis causing genospecies display unique evolutionary trajectories. *Mol. Ecol.* <https://doi.org/10.1111/mec.16805>.
- Ruzic-Sabljić, E., Lotric-Furlan, S., Maraspin, V., Cimperman, J., Logar, M., Jurca, T., Strle, F., 2006. Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in MKP and BSK-II medium. *Int. J. Med. Microbiol.* 296 (Suppl. 40), 267–273. <https://doi.org/10.1016/j.ijmm.2006.01.005>.
- Ruzic-Sabljić, E., Maraspin, V., Cimperman, J., Strle, F., Lotric-Furlan, S., Stupica, D., Cerar, T., 2014. Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in two different culture media, MKP and BSK-H. *Clin. Microbiol. Infect.* 20, 636–641. <https://doi.org/10.1111/1469-0691.12457>.
- Schulte-Spechtel, U., Fingerle, V., Goettner, G., Rogge, S., Wilske, B., 2006. Molecular analysis of decorin-binding protein A (DbpA) reveals five major groups among European *Borrelia burgdorferi* sensu lato strains with impact for the development of serological assays and indicates lateral gene transfer of the *dbpA* gene. *Int. J. Med. Microbiol.* 296 (Suppl. 40), 250–266.
- Schwartz, I., Margos, G., Casjens, S.R., Qiu, W.G., Eggers, C.H., 2021. Multipartite genome of Lyme disease *Borrelia*: structure, variation and prophages. *Curr. Issues Mol. Biol.* 42, 409–454. <https://doi.org/10.21775/cimb.042.409>.
- Stanek, G., Fingerle, V., Hunfeld, K.P., Jaulhac, B., Kaiser, R., Krause, A., Kristoferitsch, W., O'Connell, S., Ornstein, K., Strle, F., Gray, J., 2010. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. *Clin. Microbiol. Infect.* 17, 69–79.
- Stevenson, B., Miller, J.C., 2003. Intra- and interbacterial genetic exchange of Lyme disease spirochete *erp* genes generates sequence identity amidst diversity. *J. Mol. Evol.* 57, 309–324.
- Stevenson, B., Zuckert, W.R., Akins, D.R., 2000. Repetition, conservation, and variation: the multiple cp32 plasmids of *Borrelia* species. *J. Mol. Microbiol. Biotechnol.* 2, 411–422.
- Stevenson, B., El-Hage, N., Hines, M.A., Miller, J.C., Babb, K., 2002. Differential binding of host complement inhibitor factor H by *Borrelia burgdorferi* *Erp* surface proteins: a possible mechanism underlying the expansive host range of Lyme disease spirochetes. *Infect. Immun.* 70, 491–497. <https://doi.org/10.1128/IAI.70.2.491-497.2002>.
- Strube, C., Montenegro, V.M., Epe, C., Eckelt, E., Schnieder, T., 2010. Establishment of a minor groove binder-probe based quantitative real time PCR to detect *Borrelia burgdorferi* sensu lato and differentiation of *Borrelia spielmanii* by *ospA*-specific conventional PCR. *Parasit. Vectors* 3, 69.
- Takano, A., Nakao, M., Masuzawa, T., Takada, N., Yano, Y., Ishiguro, F., Fujita, H., Ito, T., Ma, X., Oikawa, Y., Kawamori, F., Kumagai, K., Mikami, T., Hanaoka, N., Ando, S., Honda, N., Taylor, K., Tsubota, T., Konnai, S., Watanabe, H., Ohnishi, M., Kawabata, H., 2011. Multilocus sequence typing implicates rodents as the main reservoir host of human-pathogenic *Borrelia garinii* in Japan. *J. Clin. Microbiol.* 49, 2035–2039.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739. <https://doi.org/10.1093/molbev/msr121>.
- Taragel'ova, V., Koci, J., Hanincova, K., Kurtenbach, K., Derdakova, M., Ogden, N.H., Literak, I., Kocianova, E., Labuda, M., 2008. Blackbirds and song thrushes constitute a key reservoir of *Borrelia garinii*, the causative agent of borreliosis in Central Europe. *Appl. Environ. Microbiol.* 74, 1289–1293.
- Tilly, K., Bestor, A., Jewett, M.W., Rosa, P., 2007. Rapid clearance of Lyme disease spirochetes lacking *OspC* from skin. *Infect. Immun.* 75, 1517–1519.
- Tyler, S., Tyson, S., Dibernardo, A., Drebot, M., Feil, E.J., Graham, M., Knox, N.C., Lindsay, L.R., Margos, G., Mechai, S., Van Domselaar, G., Thorpe, H.A., Ogden, N.H., 2018. Whole genome sequencing and phylogenetic analysis of strains of the agent of Lyme disease *Borrelia burgdorferi* from Canadian emergence zones. *Sci. Rep.* 8, 10552. <https://doi.org/10.1038/s41598-018-28908-7>.
- 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>.
- Wywiał, E., Haven, J., Casjens, S.R., Hernandez, Y.A., Singh, S., Mongodin, E.F., Fraser-Liggett, C.M., Luft, B.J., Schutzer, S.E., Qiu, W.G., 2009. Fast, adaptive evolution at a bacterial host-resistance locus: the PFam54 gene array in *Borrelia burgdorferi*. *Gene* 445, 26–37. <https://doi.org/10.1016/j.gene.2009.05.017>.