Received: 26 May 2021 | Revised: 25 October 2022 | Accepted: 30 November 2022

DOI: 10.1111/mec.16805

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Out of Asia? Expansion of Eurasian Lyme borreliosis causing genospecies display unique evolutionary trajectories

Robert E. Rollins[1,2] | Kozue Sato[3] | Minoru Nakao[4] | Mohammed T. Tawfeeq[1,5,6,7] | Fernanda Herrera-Mesías[1,8,9] | Ricardo J. Pereira[2,10] | Sergey Kovalev[11] | Gabriele Margos[12] | Volker Fingerle[12] | Hiroki Kawabata[3] | Noémie S. Becker[2]

[1]Institute of Avian Research "Vogelwarte Helgoland", Wilhelmshaven, Germany

[2]Division of Evolutionary Biology, Faculty of Biology, Ludwig-Maximilians Universität, Munich, Germany

[3]Department of Bacteriology I, National Institute for Infectious Disease, Tokyo, Japan

[4]Department of Parasitology, Asahikawa Medical University, Asahikawa, Japan

[5]VIB - KU Leuven Center for Microbiology, Leuven, Belgium

[6]CMPG Laboratory of Genetics and Genomics, Department M2S, KU Leuven, Leuven, Belgium

[7]Leuven Institute for Beer Research (LIBR), Leuven, Belgium

[8]Department of Animal Ecology, Evolution, and Biodiversity, Ruhr-Universität Bochum, Bochum, Germany

[9]Musée National d'histoire Naturelle de Luxembourg, Luxembourg, Luxembourg

[10]Department of Zoology, State Museum of Natural History Stuttgart, Stuttgart, Germany

[11]Laboratory of Molecular Genetics, Department of Biology, Ural Federal University, Yekaterinburg, Russia

[12]National Reference Center for Borrelia, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany

Correspondence
Robert E. Rollins, Institute of Avian Research "Vogelwarte Helgoland", An der Vogelwarte 21, 26386 Wilhelmshaven, Germany.
Email: robert.rollins@ifv-vogelwarte.de

Noémie S. Becker, Division of Evolutionary Biology, Faculty of Biology, LMU Munich, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany.
Email: nbecker@bio.lmu.de

Funding information
AMED, Grant/Award Number: JP20fk0108068 and JP20wm0225016; Deutsche Forschungsgemeinschaft, Grant/Award Number: BE 5791/2-1; Robert-Koch Institut

Handling Editor: Sebastien Calvignac-Spencer

Abstract

Vector-borne pathogens exist in obligate transmission cycles between vector and reservoir host species. Host and vector shifts can lead to geographic expansion of infectious agents and the emergence of new diseases in susceptible individuals. Three bacterial genospecies (*Borrelia afzelii*, *Borrelia bavariensis*, and *Borrelia garinii*) predominantly utilize two distinct tick species as vectors in Asia (*Ixodes persulcatus*) and Europe (*Ixodes ricinus*). Through these vectors, the bacteria can infect various vertebrate groups (e.g., rodents, birds) including humans where they cause Lyme borreliosis, the most common vector-borne disease in the Northern hemisphere. Yet, how and in which order the three *Borrelia* genospecies colonized each continent remains unclear including the evolutionary consequences of this geographic expansion. Here, by reconstructing the evolutionary history of 142 Eurasian isolates, we found evidence that the ancestors of each of the three genospecies probably have an Asian origin. Even so, each genospecies studied displayed a unique substructuring and evolutionary response to the colonization of Europe. The pattern of allele sharing between continents is consistent with the dispersal rate of the respective vertebrate hosts, supporting the concept that adaptation of *Borrelia* genospecies to the host is important for pathogen dispersal. Our results highlight that Eurasian Lyme borreliosis

agents are all capable of geographic expansion with host association influencing their dispersal; further displaying the importance of host and vector association to the geographic expansion of vector-borne pathogens and potentially conditioning their capacity as emergent pathogens.

KEYWORDS
Borrelia burgdorferi, geographic expansion, Lyme borreliosis, niche, pathogen evolution, vector adaptation

1 | INTRODUCTION

Lyme borreliosis (LB), also termed Lyme disease, is the most common vector-borne disease in the Northern hemisphere (Stanek et al., 2011; Steere et al., 2016), caused by certain genospecies of *Borrelia* bacteria (Kurtenbach et al., 2006; Margos et al., 2019; Stanek et al., 2011). These spirochete bacteria are maintained naturally in obligatory transmission cycles between tick vectors and specific vertebrate reservoir hosts (e.g., rodent or bird species; Kurtenbach et al., 2006). In certain cases, spirochetes can also infect humans, manifesting as LB, although humans are considered "dead-end" hosts as they do not aid in the further transmission of the bacteria (Kurtenbach et al., 2006; O'Keeffe et al., 2020; Steere et al., 2016). To complete their life-cycle, *Borrelia* spirochetes encode many proteins allowing them to infect both the tick vector and vertebrate host (Fraser et al., 1997; Kraiczy, 2016; Schwartz et al., 2021). The *Borrelia* genome is highly fragmented consisting of a linear chromosome (0.9 Mb) and up to or over 20 unique linear and circular plasmid replicons (0.6 Mb; Fraser et al., 1997; Schwartz et al., 2021). The plasmids themselves are more variable, whereas the majority of the chromosome (>93%) is stable and shows a high degree of synteny between genospecies and therefore can be used to reconstruct the evolutionary history of these bacteria (Becker et al., 2020; Schwartz et al., 2021; Walter et al., 2017).

In North America, human LB is predominantly caused by *Borrelia burgdorferi* sensu stricto (s.s.) while three additional genospecies act as main causative agents across Eurasia: *Borrelia afzelii*, *Borrelia bavariensis*, and *Borrelia garinii* (Kurtenbach et al., 2006; Margos et al., 2019; Stanek et al., 2011). *Borrelia* genospecies cannot transmit successfully through all tick species (Eisen, 2020; Margos et al., 2019) and the majority of species can only use specific vertebrate classes as reservoir hosts (i.e., rodents, birds, etc.; Kurtenbach et al., 2006; Margos et al., 2019; O'Keeffe et al., 2020; Wolcott et al., 2021). For this reason, the evolutionary history and geographical distribution of these bacteria is inherently linked to which tick species they can use as vector and which animals as reservoir hosts (Kurtenbach et al., 2006; O'Keeffe et al., 2020). Chromosome level analysis did unravel the spread of *B. burgdorferi* s.s. across North America, showing that spirochetes are able to migrate over large geographic distances most likely due to host movement (i.e., bird association) but also showed minimal structure between different North American tick species (i.e., *Ixodes scapularis* and *Ixodes pacificus*; Hoen et al., 2009; Tyler et al., 2018; Walter et al., 2017). *Borrelia burgdorferi* s.s. is a generalist spirochete using multiple host classes (i.e., rodents, birds) as reservoir hosts and does not appear to structure between North American tick vectors, which limits the ability of this system to disentangle the role individual host and/or vector association could have on the evolutionary history of these bacteria. Here however, the Eurasian genospecies (*B. afzelii*, *B. bavariensis*, *B. garinii*) offer a unique opportunity to study these factors as each genospecies display specific vertebrate reservoir host associations and also transmit successfully through multiple tick transmission cycles (Kurtenbach et al., 2006; O'Keeffe et al., 2020).

Both *B. afzelii* and *B. bavariensis* utilize rodents as reservoir hosts, whereas *B. garinii* is adapted to avian host species (Kurtenbach et al., 2006; Wolcott et al., 2021; Figure 1). These associations are thought to modify the rate at which *Borrelia* spirochetes are able to disperse with rodent-associated *Borrelia* dispersing at lower rates in comparison to bird-adapted genospecies (Kurtenbach et al., 2006; O'Keeffe et al., 2020). Studies utilizing multiple locus sequence typing (MLST), which is a genotyping scheme based on eight chromosome located housekeeping genes (Margos et al., 2008), have shown that rodent-adapted genospecies do show increased spatial structuring in comparison to bird-adapted genospecies (Gallais et al., 2018; Norte et al., 2020; Vollmer et al., 2011, 2013). These studies though were based on MLST data and focused predominantly on European isolates. Research on *B. burgdorferi* s.s. in North America showed that utilizing whole genome sequencing was able to detect patterns of population dispersal on a more refined scale than based on MLST analysis alone (Hoen et al., 2009; Walter et al., 2017). This highlights the need to integrate genome level analyses on the Eurasian genospecies including Asian isolates to corroborate the results of MLST-based studies and clarify the role host association plays across the entire range of these genospecies.

In addition to variable host-association types, Eurasian *Borrelia* genospecies currently exist in separate terrestrial transmission cycles vectored predominately by two generalist tick species: *Ixodes persulcatus* in Asia and *Ixodes ricinus* in Europe (Kurtenbach et al., 2006; Figure 1). In comparison to *B. burgdorferi* s.s. which did not show clustering based on tick vector, chromosome-level analysis of *B. bavariensis* found a distinct split between the European and Asian isolates, with evidence for the European population having undergone a demographic bottleneck (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2019). This bottleneck is attributed to

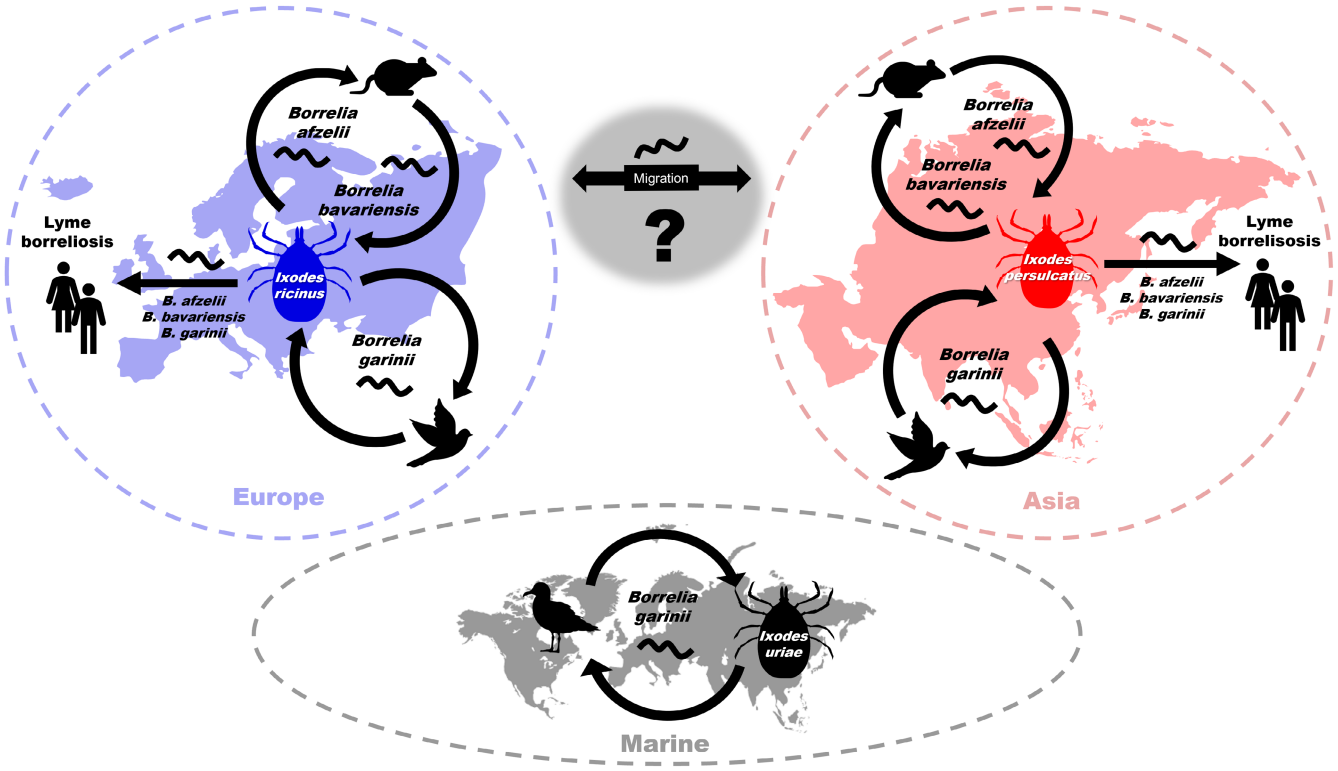


FIGURE 1 Schematic overview of the transmission cycles of *B. afzelii*, *B. bavariensis*, and *B. garinii* across Eurasia. These three *Borrelia* genospecies are maintained predominately by the tick vector *I. ricinus* in Europe and *I. persulcatus* in Asia in a transmission cycle utilizing either rodents (*B. afzelii* and *B. bavariensis*) or birds (*B. garinii*) as reservoir hosts (Kurtenbach et al., 2006; Margos et al., 2011, 2019). *Borrelia garinii* specifically utilizes interconnected terrestrial and marine based transmission cycles (Comstedt et al., 2006, 2009, 2011). In marine systems, this species is maintained by seabird reservoir host species and the vector *I. uriae* (Comstedt et al., 2011). In both Europe and Asia, all three genospecies can be transmitted to humans through *I. ricinus* or *I. persulcatus* and can manifest as Lyme borreliosis (Kurtenbach et al., 2006; Stanek et al., 2011)

invading a novel tick vector (*I. ricinus*) from an ancestral Asian population (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2013, 2019). Whether or not the other genospecies also underwent this westward colonization pattern is currently not known and warrants further study. An important factor to consider here is if ancestral tick diversification could have influenced the observed pattern within *B. bavariensis*. Recent analysis based on full transcriptome sequencing of various tick species showed that *I. ricinus* and *I. persulcatus* are not sister taxa (Charrier et al., 2019). Instead, this analysis placed *I. persulcatus* in a monophyletic clade with the main North American LB tick vector (*I. scapularis*). This structuring suggests a potential eastward migration of tick species which would be incongruent with the proposed Asian origin and westward migration of *B. bavariensis*. Even so, both *Borrelia* and tick phylogenies remain undated and these transcriptomic analyses lack many Asian tick species (Charrier et al., 2019), limiting the ability to determine what role tick diversification could have played in *Borrelia* evolution and whether the evolution of the different *Borrelia* genospecies predates tick speciation or not.

Regardless though, the Eurasian *Borrelia* genospecies have each successfully established into different tick vectors and further differ in their host associations, creating an opportunity to disentangle how these associations have and could further influence the evolutionary history of these bacteria. Genomic and MLST analyses have shown that there are evolutionary consequences to reservoir host or vector association. Yet, no study to date has integrated genomic data from all three Eurasian-distributed genospecies and, in general, there is a lack of whole genome sequences of Asian isolates. Previous studies have hypothesized that *B. bavariensis* indeed originated in Asia (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2019) which we hypothesize to be true for the other two genospecies (*B. afzelii*, *B. garinii*) which share a Eurasian distribution. Herein we report the reconstructed evolutionary history of 142 *B. afzelii*, *B. garinii*, and *B. bavariensis* Eurasian isolates based on full chromosome sequences, including the first Japanese *B. afzelii* genomes sequenced and wild-caught samples from all three genospecies. Our results highlight that these genospecies share an Asian origin with support for expansion out of an ancestral Asian population into Europe. Post-colonization gene flow appears to be associated with the dispersal range of the respective reservoir host species. Our results provide new information on the ability of three *Borrelia* genospecies to colonize new environments, defined by vector and reservoir host species, and how this could relate to the further expansion of pathogenic *Borrelia* genospecies capable of causing human disease.

2 | MATERIALS AND METHODS

2.1 | Isolates, culturing, and DNA extraction

The major aim of this study was to estimate the direction of colonization in each *Borrelia* genospecies between Asia and Europe. To achieve this though, we needed to create an isolate library of the three genospecies including isolates arising from wild-caught ticks, where possible. Therefore, this study utilized DNA of 136 Eurasian *Borrelia* isolates coming from *B. afzelii* (Asian, $n = 20$; European, $n = 13$), *B. bavariensis* (Asian, $n = 27$; European, $n = 19$), and *B. garinii* (Asian, $n = 25$; European, $n = 32$). Of these, 52 are novel *Borrelia* isolated from wild-caught ticks collected either in Japan ($n = 43$) or Germany ($n = 9$) in the years 2018–2019 (see Supporting Information S1 and Tables S2–S4–). Additionally, 55 European isolates (*B. afzelii*, $n = 11$; *B. garinii*, $n = 25$; and *B. bavariensis*, $n = 19$) and 12 Japanese isolates (*B. bavariensis*, $n = 8$; *B. garinii*, $n = 4$) were provided by the German National Reference Center for *Borrelia* at the Bavarian Food and Health Safety Authority and the National Institute of Infectious Disease, respectively. These additional isolates predominantly come from LB patients ($n = 59$) with a few isolated from wild-caught ticks ($n = 8$). Finally, Russian *B. bavariensis* ($n = 6$; Becker et al., 2020) and *B. garinii* ($n = 11$) isolates arising from wild-caught ticks were included in the study (see Supporting Information S1). For three isolates (UO2, UO3, UO4), the source material is not known. For all information on isolates, including origin and source material, refer to Table S1.

Borrelia isolates were cultured either in inhouse-made MKP (Preac-Mursic et al., 1986) (all European isolates) or inhouse-made BSK-H (Pollack et al., 1993; Takano et al., 2014) (all Russian and Japanese isolates) medium according to standard procedures (Pollack et al., 1993; Preac-Mursic et al., 1986), until the cultures reached a density of at least 10^8 cells per ml, at which point whole genomic DNA was extracted. Genomic DNA from all European isolates was extracted using a Maxwell 16 LED DNA kit (Promega) and from all Japanese and Russian isolates using the Wizard Genomic DNA purification kit (Promega). DNA quality (260/280) and concentration were measured using a NanoDrop 1000 photometer (Thermo Fisher Scientific) and a Qubit 3.0 fluorometer (Thermo Fisher Scientific), respectively.

Previously isolated samples provided by collaboration partners were assigned to genospecies through amplifying and sequencing the eight MLST genes (Margos et al., 2008). Isolates produced during this study were assigned to genospecies through amplifying one MLST gene, *recG*, and by comparing this sequence to the PubMLST database for *Borrelia* (https://pubmlst.org/borrelia). The isolate was assigned to the closet allele type match. This approach has been used in previous studies reliably to assign *Borrelia* genospecies (e.g., Rollins et al., 2021). For further details regarding isolation and processing see Supporting Information S1.

2.2 | Whole genome sequencing, assembly, and determining orthologous genes

Within the course of this study, we aimed to reconstruct the evolutionary history of *B. afzelii*, *B. bavariensis*, and *B. garinii* for which we needed to focus on the portions of the genome which are stable and homologous across all isolates. For this, we aimed to reconstruct full chromosome sequences through whole genome sequencing for each isolate, as the chromosome represents a large proportion of the *Borrelia* genome and the majority of the sequence (>93%) is stable (Schwartz et al., 2021; Walter et al., 2017). For all samples, libraries were produced according to the Nextera XT sample preparation guide (Illumina). Library quality was checked using an Agilent TapeStation 2200 (Agilent) before being sequenced using an Ilumina MiSeq platform according to standard protocol (Illumina) that produced paired-end reads of 250 bp. Illumina reads were first trimmed for Illumina MiSeq adapter sequences using Trimmomatic version 0.38 (Bolger et al., 2014a, 2014b) before being assembled using SPAdes version 3.13.0 (Bankevich et al., 2012), which has been shown to be the best option for de novo assemblies of *Borrelia* genomes (Becker et al., 2020). Pacific Bioscience sequences were obtained for three *B. bavariensis* isolates (PBi, A104S, and NT24; Becker et al., 2020) and three *B. garinii* isolates (PHeI, PBr, and NT31; see Supporting Information Methods). Additionally, three *B. afzelii* chromosomes were downloaded from GenBank for use as references and inclusion in all analyses: PKo (CP009058.1), K78 (CP002933.1), and ACA-1 (NZ_ABCU00000000.2). SPAdes contigs were then mapped to reference chromosomes using NUCmer version 3.23 from the package MUMmer (Delcher et al., 2002; Kurtz et al., 2004). Final chromosomes were produced according to the mapping protocol outlined in Becker et al. (2020; see Supporting Information Methods). Three additional *B. bavariensis* chromosomes were downloaded from GenBank and used in further analyses: SZ (CP007564.1), BgVir (CP003151.1), and NWJW1 (CP003866.1).

Final chromosomes were uploaded and annotated using the RAST annotation server (Aziz et al., 2008; Overbeek et al., 2014) for which proposed coding sequences were extracted. Orthologous sequences were determined by using the CRBHits package (Ullrich, 2020) as implemented in R (R Core Team, 2019). Briefly, all coding sequences for each chromosome were compared pairwise to all other chromosomes using the crb2rbh function. This information was fed into the integrated DAGchainer (Haas et al., 2004) command to create links between syntenous genes. Synteny member groups were then determined using the "cluster_infomap" command as implemented from the igraph R package (Csárdi & Nepusz, 2006) on a matrix of gene names and DAGchainer links. FASTA files for all synteny member groups were generated and aligned using MUSCLE (Edgar, 2004a, 2004b) for all gene copies and each genospecies. For each alignment, nucleotide diversity (π) (Nei, 1987) was estimated in R (R Core Team, 2019) using the package pegas (Paradis, 2010)

as well as the proportion of isolates carrying the synteny member group.

2.3 | Recombination analysis

Recombination is known to be low on the *Borrelia* chromosome (Gatzmann et al., 2015; Schwartz et al., 2021; Walter et al., 2017). Even so, recombinant areas of the genome are inherited through horizontal gene transfer (Arnold et al., 2022) and could therefore bias phylogenetic reconstruction. For this reason, we aimed to identify recombinant regions along the chromosome to remove prior to phylogenetic reconstruction but also to determine what genes may be influence by recombination in our data set. Final assembled chromosomes and GenBank sequences ($n = 142$) were aligned using MAFFT version 7.407 (Katoh et al., 2002; Katoh & Standley, 2013). To determine recombinant regions along the chromosomes, we applied the four-gamete condition (Hudson & Kaplan, 1985) to the full chromosome alignment, as described in Gatzmann et al. (2015).

The ordered list of segregating sites along the chromosome was divided into blocks containing the same number of SNPs ($n = 12$). Each pair of SNPs in each block was then assessed if the four-gamete condition was violated or not. The within block average and standard deviation was then calculated and averaged across all blocks and used as a measure of background violation due to double hits or back mutations. To single out SNP blocks which were most likely under recombination, we then calculated all pairwise comparisons between blocks and recorded the violation score. This score was then averaged over all comparisons for a specific block. Blocks were considered recombinant if:

$$x_i \geq \mu_{\text{within}} + 2sd_{\text{within}},$$

and nonrecombinant if:

$$x_i < \mu_{\text{within}} + 2sd_{\text{within}},$$

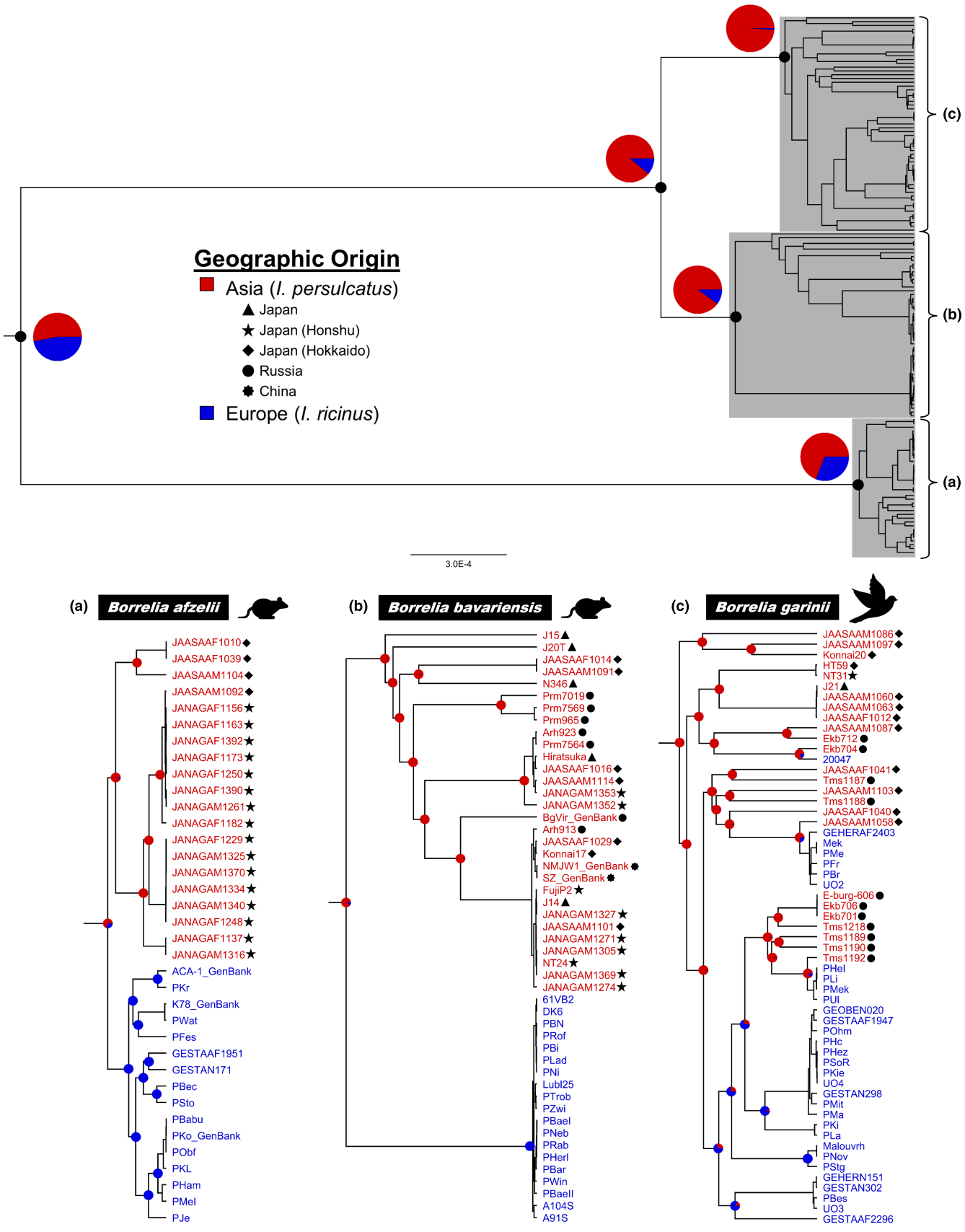
where x_i is the average violation of block i over all comparisons, μ_{within} is the average within-block violation score, and sd_{within} is the standard deviation of within-block violation score.

We ran the analysis seven times using each time an alignment containing one, two or all genospecies. In this way, all crosswise comparisons were accounted for. The proportion of recombinant blocks was calculated for each comparison as the number of blocks meeting the above requirement divided by the total number of SNP blocks for the alignment in question. Recombinant blocks were extracted and compared using BLAST version 2.8.1 (Altschul et al., 1990; Camacho et al., 2009) (algorithm: blastn) to the full chromosome for each isolate. BLAST start and end positions were then compared to the RAST annotation files to determine if the recombining region fell within a coding (synteny member group) or noncoding region of the chromosome. Synteny member groups identified as being influenced by recombinant SNP blocks in all crosswise comparisons were considered to be influenced by horizontal gene transfer and were analysed phylogenetically in BEAST version 2.6.6 (Bouckaert et al., 2019) with the following parameters: coalescent model with constant size, strict clock (Drummond et al., 2006) with a clock-rate fixed to 1, GTR substitution model with four gamma categories (Tavaré, 1986). Potential recombination events were checked by plotting individual gene phylogenies against the full chromosome tree produced without potential recombining regions (see following methods section), using the "cophylo" command in the R package PHYTOOLS (Revell, 2012).

2.4 | Phylogenetic reconstruction including biogeographical inference

In order to estimate the pattern of *Borrelia* colonization across Eurasia, we needed to estimate the probability of each ancestral to have a European or Asian origin. This required phylogenetic reconstruction considering biogeographical inference for which we could only use homologous, nonrecombining portions of the chromosome. For this, chromosome regions identified as recombinant in the previous described analysis were removed and the sequences were realigned using MAFFT version 7.407 (Katoh et al., 2002; Katoh & Standley, 2013; final alignment length: 936,908 bp). Phylogeny reconstruction was performed in BEAST version 2.6.6 (Bouckaert et al., 2019) with the following parameters: coalescent model with constant size, strict clock (Drummond et al., 2006) with a clock-rate fixed to 1, GTR substitution model with four gamma categories (Tavaré, 1986). Geographic location was modelled as a discrete trait (Europe or Asia) and included in the phylogenetic inference utilizing the default settings for a discreate character (i.e., symmetric

FIGURE 2 Phylogeny of *B. afzelii*, *B. bavariensis*, and *B. garinii* based on the main chromosome corrected for recombining regions (see methods). Top panel displays the full phylogenetic tree with shaded clades displaying individual genospecies shown in detail in a) *Borrelia afzelii*, b) *Borrelia bavariensis*, and c) *Borrelia garinii*. Phylogeny reconstruction was performed in BEAST version 2.6.6 (Bouckaert et al., 2019) with the following parameters: Coalescent model with constant size, strict clock (Drummond et al., 2006) with a clock-rate fixed to 1, GTR substitution model with four gamma categories (Tavaré, 1986). Geographic location was modelled as a discrete trait (Europe or Asia) and included in the phylogenetic inference utilizing the default settings for a discreate character (i.e., symmetric mutation death model with one gamma category; Lemey et al., 2009; Wallace et al., 2007). Probablities for a given geographic origin are given at each internal node in the full phylogeny and each genospecies specific phylogeny (a-c). Three independent runs were performed each with 50 million steps chain with a relative burnin of 20% before selecting the best tree with TreeAnnotator version 1.10.4 (Drummond & Rambaut, 2007). Convergence of parameters was checked with Tracer version 1.7.1 (Rambaut et al., 2018). Colours correspond to geographic location: Europe (blue) and Asia (red). Pie charts display the inferred probability of the ancestral at this node to either exist in Asia (red) or Europe (blue). Shapes plotted next to isolates refer to geographic locations within Asia



mutation death model with one gamma category; Lemey et al., 2009; Wallace et al., 2007). Three independent runs were performed each with 50 million steps chain with a relative burnin of 20% before selecting the best tree with TreeAnnotator version 1.10.4 (Drummond & Rambaut, 2007). Convergence of parameters was checked with Tracer version 1.7.1 (Rambaut et al., 2018). Convergence to a single topology in all three independent runs was checked manually in FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), which was also used to plot the tree shown in Figure 2.

2.5 | Population genetic analyses

An additional aspect in terms of estimating the pattern of colonization between Asia and Europe was to test how genetic diversity within each genospecies varied between the continents. The aim was to better understand how proposed host and vector associations have potentially influenced the evolution and dispersal of these bacteria. We further extend this to ask if the inclusion of nonrandom samples could bias population genetic statistics as our data set includes nonrandomly sampled isolates (predominantly isolated from LB patients) but also randomly sampled isolates arising from wild-caught ticks. All statistical analysis was performed in R version 3.6.1 (R Core Team, 2019). Genetic diversity (π) (Nei, 1987) and Tajima's *D* test statistic (Tajima, 1989) were estimated in the package pegas (Paradis, 2010). Analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed using the package poppr (Kamvar et al., 2015) whereas F_{ST} (Nei, 1987) and D_{XY} (Hudson et al., 1992) were estimated with the package PopGenome (Pfeifer et al., 2014).

2.6 | Identification of plasmid content through plasmid partitioning genes

The primary aim of this study was to utilize full chromosome sequences to estimate the direction of *Borrelia* colonization between Europe and Asia as well as quantify the variability in genetic diversity between populations. Even so, the *Borrelia* genome contains many accessory plasmid types which have also been shown to be influenced by demographic events such as the bottleneck observed in *B. bavariensis* where the overall diversity of plasmid types also decreased due to colonizing a novel tick vector (Becker et al., 2020). To determine if this was also the case for the other two Eurasian genospecies, we estimated plasmid content based on the number of unique plasmid partitioning genes present in each assembly. These partitioning genes have been shown to be plasmid-specific and exist as single copies in *Borrelia* isolates (Casjens et al., 2012; Casjens & Huang, 1993; Fraser et al., 1997). Identification of plasmid partitioning genes was performed as outlined in Becker et al. (2020; see Supporting Information Methods). Briefly, we used BLAST version 2.8.1 (Altschul et al., 1990; Camacho et al., 2009; algorithm: blastn) to search for the presence of plasmid partitioning genes of the PFam32, 49, 50, and 57.62 families in the assembled SPAdes contigs. Hits were removed if they did not cover more than half the length of the references and had lower than 80% identity. After curation, we defined a plasmid being present if at least one of the partitioning genes was present in the assembled contigs.

Standard two-side, unpaired *t*-tests were run on plasmid number between genospecies comparing the two geographic populations using the function t.test from the BASE R package (R Core Team, 2019). Classical multidimensional scaling (MDS) was run using the cmdscale function using the base R package on a distance matrix calculated from the binary presence/absence plasmid data per isolate. Further effects on plasmid content were tested using a generalized linear mixed effects model assuming a Poisson error distribution using the glmer function from the package LME4 (Bates et al., 2015). Fixed effects were included for sample origin (Asia vs. Europe) and source (human vs. tick isolate) and genospecies was fitted as a random effect. Mean estimates and their 95% credible intervals were estimated based on 5000 simulations using the sim function from the package arm (Gelman & Su, 2016). Residual error was calculated according to Nakagawa and Schielzeth (2010).

3 | RESULTS

In total, 142 full chromosome sequences were used for all genetic analyses, of which 136 were assembled de novo from Illumina MiSeq data. Final chromosome length ranged from 825.7 to 906.2 kb (mean: 900.0 kb) with chromosome coverage varying from 8 to 572x (mean: 104.6x; Table S1). Among all isolates, 1092 chromosome synteny member groups were identified, with 807 synteny member groups found in at least 80% of isolates ($n = 114$), but only 529 synteny member groups found in all isolates ($n = 142$) (Table S5). Genes with the highest diversity among genospecies were predominately annotated as "hypothetical proteins" (Table S6), while the most conserved genes were predominately related to general housekeeping functions (Table S6). Most isolates under study were assigned to an existing *recG* allele type, with 10 isolates differing by one ($n = 2$), two ($n = 4$), three ($n = 3$), or four ($n = 1$) allelic positions (Table S1). All genospecies assignments based on *recG* sequence were confirmed through phylogenetic reconstruction based on full chromosome sequences (Figure 2).

Based on the four-gamete test used to identify recombining SNP blocks along the chromosome, within-genospecies comparisons generally showed a higher proportion of recombining blocks relative to that observed among-genospecies (*B. afzelii*, prop = 0.05; *B. garinii*, prop = 0.05; Table 3), with the notable exception of *B. bavariensis* (prop = 0.009; Table 3). Only in the pairwise comparison of *B. afzelii* and *B. bavariensis* was the proportion of recombining blocks on the same magnitude as within-genospecies comparisons (prop = 0.02; Table 3). Four synteny member groups were identified independently as being influenced by recombination (SM407-409 & SM730; Table 4). Gene specific phylogenies showed horizontal gene transfer predominantly occurred within-genospecies and among isolates arising from the same continent (Figures S1–S4). *Borrelia*

garinii was the only genospecies where horizontal gene transfer between isolates arising from different continents was observed (Figures S1–S4). Additionally, only one of the four synteny member groups (SM730) displayed evidence for horizontal gene transfer among-genospecies; with potential recombination between *B. bavariensis* and *B. garinii* (Figure S4). In all cases, horizontal gene transfer was inferred when an isolates position in the gene phylogeny did not match the chromosome phylogeny (Figures S1–S4).

The ancestral node for *B. garinii* separated a clade containing only Asian isolates from a clade containing isolates from both continents (Figure 2a). In both *B. afzelii* and *B. bavariensis* isolates from the two continents formed monophyletic clades (Figures 2a,b) which was not the case for *B. garinii* (Figure 2c). In the case of *B. bavariensis*, a deep branching event was observed with the European isolates being characterized by a low divergence and almost clonal population (Figure 2b), which was not observed in the other genospecies (Figure 2a,c). Asian *B. afzelii* isolates coming from Honshu and Hokkaido formed two reciprocally monophyletic clades, except for one Hokkaido isolate belonging to the Honshu clade (Figure 2a). In comparison, Asian *B. bavariensis* and *B. garinii* isolates did not form monophyletic clades based on geographic origin (Figure 2b). For *B. garinii*, this trend extended to monophyletic clades containing isolates from both continents (Figure 2c). For all three genospecies, the phylogeographic reconstruction inferred a higher probability of the ancestral of each genospecies to be in Asia (*B. afzelii*, 0.69; *B. bavariensis*, 0.91; *B. garinii*, 0.99; Figure 2), consistent with an Asian origin followed by an expansion towards Europe for all three genospecies. Naturally, the geographic origin of the ancestor of the three genospecies could not be inferred. Furthermore, according to the ultrametric tree the oldest split was observed in *B. bavariensis*, followed by *B. garinii*, and with the most recent colonization event occurring in *B. afzelii*.

Higher genetic diversity (π) was found in Asian *B. bavariensis* and *B. garinii* in comparison to their European counterparts (Table 1). Genetic diversity was similar between Asian and European *B. afzelii* isolates (Table 1). In all cases, the *Borrelia* populations showed negative Tajima's *D* values (Table 1), but the European samples always showed more negative values (Table 2). *Borrelia bavariensis* displayed the largest absolute divergence value (D_{xy}) and *B. afzelii* the lowest (Table 1). *Borrelia bavariensis* displayed the strongest geographic differentiation between the European and Asian samples ($F_{ST} = 0.744$; $AMOVA_{continent}$ = 69.7% of molecular variance (σ)) followed by *B. afzelii* ($F_{ST} = 0.570$; $AMOVA_{continent}$ = 40.2% of σ; Tables 1 and 2). Regions (defined as country or sampling locality if known, Table S1 and S3) within continents further explained a higher proportion of genetic variation in *B. afzelii* samples ($AMOVA_{Region}$ = 23.6% of σ; Table 2) and differentiation was observed between randomly sampled *B. afzelii* isolates from the islands of Hokkaido (ASA) and Honshu (NAG) ($F_{ST} = 0.379$; Table S7). However, this was not observed for *B. bavariensis* ($AMOVA_{Region}$ = 0.99% of σ; Table 2) and, indeed, randomly sampled *B. bavariensis* isolates from the islands of Hokkaido and Honshu did not show geographic differentiation ($F_{ST} = 0.057$; Table S7). Less geographic differentiation by continent was observed in *B. garinii* ($F_{ST} = 0.13$; $AMOVA_{Continent}$ = 8.7% of σ; Tables 1 and 2). Nucleotide diversity did not differ between randomly ($\pi_{Europe} = 0.00633$; $\pi_{Asia} = 0.0095$; Table S7) and nonrandomly ($\pi_{Europe} = 0.00619$; $\pi_{Asia} = 0.00900$; Table 2) sampled *B. garinii* isolates. A similar trend for F_{ST} of *B. garinii* populations was observed for random ($F_{ST} = 0.1003$; Table S7) and nonrandom ($F_{ST} = 0.1318$; Table 1) samples. For *B. afzelii* and *B. bavariensis* we report random samples coming from two distinct sampling locations within Japan, and this does show that there can be local level variation in population level statistics (Table S7).

Borrelia afzelii and *B. bavariensis* both differed significantly in plasmid numbers between Europe and Asia, but in *B. afzelii* the European population had more plasmids compared to the Asian population (two-sided unpaired *t*-test, $p = .03$) whereas the reverse pattern was observed for *B. bavariensis* ($p < .001$; Figure 3a). *Borrelia garinii* populations did not differ in overall plasmid content ($p = .08$) but had significantly fewer plasmids in comparison to both *B. afzelii* populations (Asian, $p = .003$; European, $p < .001$) and to Asian *B. bavariensis* ($p < .001$; Figure 3a). Only European *B. bavariensis* showed a lower absolute plasmid number (number of unique plasmid types present in at least one isolate; black circle; Figure 3a) in comparison to the other populations and species. MDS analysis based on the plasmid profile showed that European samples represent a subset of the overall plasmid profile diversity but that plasmid profiles between genospecies do not show much differentiation (Figure 3b). In accordance with this finding, no plasmid types were more frequently associated with factors such as genospecies or geography (Figure S5). Tick isolates had on average higher plasmid content relative to human LB patient isolates (Table S8; mean: 1.19; 95% CI: 0.16, 2.22), with genospecies explaining a high proportion of variability (~46%) in plasmid content (Table S8).

TABLE 1 Population genetics statistics for full population samples of *B. afzelii*, *B. bavariensis*, and *B. garinii*. The Asian populations for *B. garinii* and *B. bavariensis* contain all Russian samples. These calculations include nonrandomly sampled isolates (both tick and human), but values calculated for randomly sampled isolates showed similar statistics (Table S7)

Genospecies	Population	*n*	π	Tajima's *D*	F_{ST}	D_{XY}
Borrelia afzelii	Asian	20	0.00193	−3.932	0.570	0.00379
	European	16	0.00217	−4.193		
Borrelia bavariensis	Asian	30	0.00784	−2.616	0.744	0.0141
	European	19	0.000170	−4.138		
Borrelia garinii	Asian	25	0.00900	−2.302	0.130	0.00694
	European	32	0.00619	−3.353		

TABLE 2 Hierarchical AMOVA (Excoffier et al., 1992) of *B. afzelii*, *B. bavariensis*, and *B. afzelii* populations coming from Europe and Asia describing the percentage of genetic variation (σ) attributed to each hierarchical level. Regions within continent (Europe, Asia) are defined as country or sampling locality if known. The Asian populations for *B. garinii* and *B. bavariensis* contain all Russian samples

Genospecies	Level	σ (%)
Borrelia afzelii	Between continents	40.223
	Regions within continent	23.612
	Within samples	36.165
Borrelia bavariensis	Between continents	69.654
	Regions within continent	0.988
	Within samples	29.358
Borrelia garinii	Between continents	8.749
	Regions within continent	9.272
	Within samples	81.979

TABLE 3 Summary of recombination analysis utilizing the four-gamete test (Hudson & Kaplan, 1985). All comparison including all, two, or individual genospecies were analysed using all chromosome sequences aligned with MAFFT (Katoh et al., 2002; Katoh & Standley, 2013). Each sequence was broken down into blocks containing an equal number of variable positions ($n = 12$) for which a violation score was calculated to determine if a block is recombinant. Recombinant blocks were then compared back to all chromosome RAST annotations to determine the recombinant block location (i.e., coding or noncoding)

Comparison	SNP blocks	Proportion recombining	Coding	Noncoding
afzelii-bavariensis-garinii	8033	0.003	0.92	0.08
afzelii-bavariensis	6416	0.02	0.93	0.07
afzelii-garinii	6733	0.006	0.93	0.07
bavariensis-garinii	4763	0.004	0.88	0.12
afzelii	784	0.05	0.91	0.09
bavariensis	2275	0.009	0.85	0.15
garinii	2616	0.05	0.93	0.07

4 | DISCUSSION

The expansion of vector-borne pathogens is inherently linked to their ability to infect and transmit through reservoir host and vector populations. This fact can be observed as three *Borrelia* genospecies (*B. afzelii*, *B. bavariensis*, and *B. garinii*) are currently vectored predominantly by two different tick species (*I. persulcatus* in Asia, and *I. ricinus* in Europe) and further differ in their reservoir host associations. This means that each of these genospecies has successfully undergone geographic expansion most likely facilitated through

TABLE 4 Synteny member groups identified in all pairwise four-gamete test (Hudson & Kaplan, 1985) comparisons

Synteny group	*n*	Annotation (RAST)
syn_mem_407	141	Two-component system sensor histidine kinase, sensory box histidine kinase/response regulator
syn_mem_408	141	Cof protein, HD superfamily hydrolase
syn_mem_409	141	DNA-3-methyladenine glycosylase II (EC 3.2.2.21)
syn_mem_730	142	Membrane proteins related to metalloendopeptidases, M23/M37 peptidase domain protein

reservoir host and vector associations. Yet, how this geographic expansion occurred and in which order was not known due to a lack of genomic data. Here, we report a reconstructed phylogeny of 142 Eurasian isolates belonging to the genospecies *B. afzelii*, *B. bavariensis*, and *B. garinii*.

Phylogenetic reconstruction inferred a higher probability of the ancestral of each genospecies to be in Asia (*B. afzelii*, 0.69; *B. bavariensis*, 0.91; *B. garinii*, 0.99; Figure 2) suggesting for the first time that all three have an Asian origin. *Borrelia bavariensis* was already argued to have an Asian origin based on genomic analysis (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2013, 2019). Previous work utilizing MLST suggested that Asian *B. afezlii* samples could be basal in the phylogeny (Takano et al., 2011; Vollmer et al., 2013) and for *B. garinii* only partial structuring between continents was previously reported (Norte et al., 2020; Vollmer et al., 2013). Utilizing full chromosome sequences though, we were able to robustly reconstruct the evolutionary history and support this unified Asian origin. This could support that successful establishment into a novel tick vector, *I. ricinus*, may have facilitated this expansion. Even so, both *Borrelia* and tick phylogenies remain undated limiting the ability to determine in what order tick speciation and expansion of *Borrelia* into Europe occurred. Even so, each colonization event of Europe happened independently in time, as the ancestral for each genospecies occurs at a different point in the time-calibrated phylogeny and showing for the first time the order in which colonization occurred (Figure 2). This highlights that each genospecies was able to independently colonize Europe and that *B. bavariensis* displays the most ancient split (Figure 2b), with *B. afzelii* colonizing Europe relatively recently (Figure 2a). This result was further supported by patterns in absolute divergence (D_{XY}) with *B. bavariensis* showing the highest value (i.e., oldest) and *B. afzelii* showing the lowest (i.e., youngest).

Based on phylogenetic reconstruction and patterns of diversity, we were also able to determine that colonization of Europe affected each genospecies differently. The observed demographic bottleneck previously found in *B. bavariensis* (Becker et al., 2020; Gatzmann et al., 2015) and seen in our phylogenetic reconstruction (Figure 2b), is a unique attribute to this genospecies. Nucleotide diversity (π) did not differ by an order of magnitude in the other genospecies (Table 1). Although all populations showed negative Tajima's *D* values (Table 2) which could indicate a recent selective sweep but,

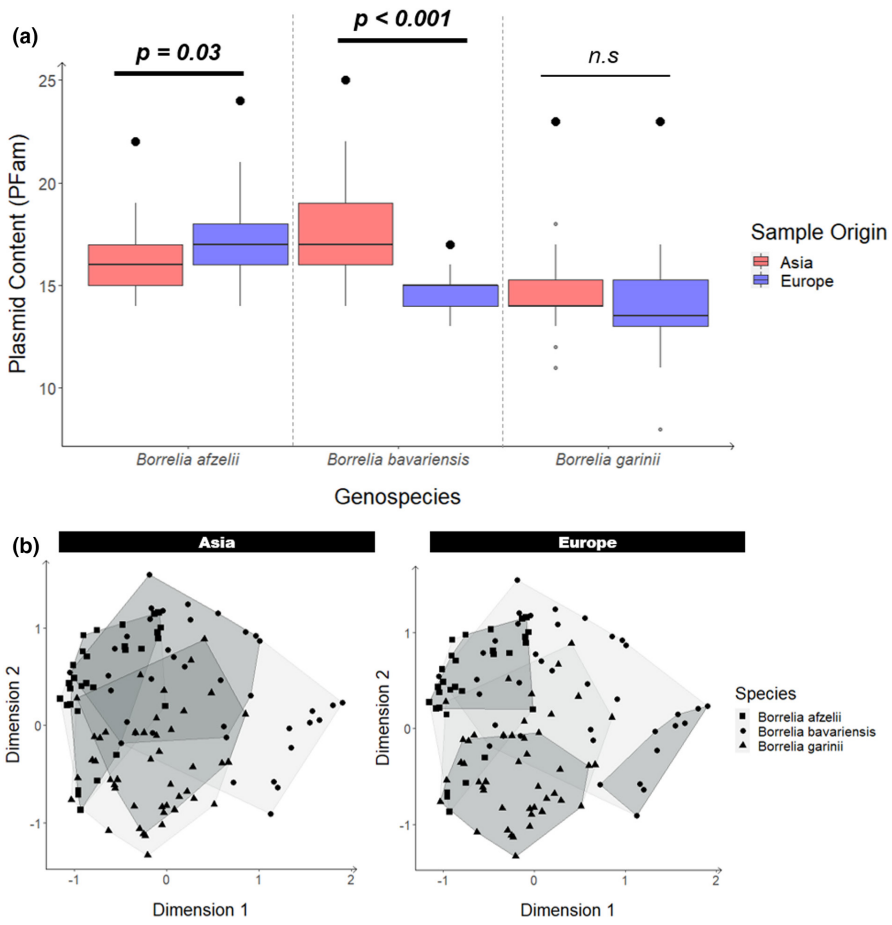


FIGURE 3 Analysis of plasmid content for sequenced strains estimated by the unique number of plasmid partitioning genes (PFam32, 49, 50, and 57.62) present in the assembled contigs. A plasmid was considered present if at least one of the partitioning genes was present. (a) Boxplot of all plasmids present in isolates from Asia or Europe. The black circles represent the absolute number of unique plasmid types found in the geographic population defined as the plasmid type being observed in at least one isolate. *p*-values refer to an unpaired, two-sided *t*-test run on plasmid number between the European and Asian populations of each species individually. (b) MDS analysis on plasmid presence/absence matrix for all samples. This figure shows the same MDS twice with emphasis on Asia (left) and Europe (right) by outlining isolates from Asia or Europe in a dark grey. Shapes correspond to genospecies: *B. afzelii* (square), *B. bavariensis* (circle), *B. garinii* (triangle)

more probably, a recent population expansion. Negative Tajima's *D* values due to the influence of population expansion would be expected for bacteria due to their asexual reproduction (Gatzmann et al., 2015; Tajima, 1989). Here though, European samples always showed more negative values (Table 2), which is in the line with a more recent or a stronger expansion into Europe. We were able to further extend this past the chromosome level by also quantifying plasmid profiles in this study based on the presence of plasmid partitioning genes in the assemblies. Here, plasmid diversity was only reduced in European *B. bavariensis* (Figure 3a) as shown in previous work (Becker et al., 2020). Even so, MDS analysis on plasmid profiles showed that the European isolates only represented a subset of all plasmid diversity (Figure 2b), but no plasmid was associated with a specific genospecies or continent (Figure S5). Future work would be required to better understand how the plasmid portion of the genome was influenced through the demographic history described in this study. These results taken together suggest that, even though all genospecies have recently expanded into Europe, only *B. bavariensis* underwent a demographic bottleneck in relation to this colonization supporting the hypothesis of a vector switch into *I. ricinus* (Becker et al., 2020; Margos et al., 2019). This could highlight a difference in niche breadth within the three genospecies. Niche here refers to a set of environmental conditions in which the members of a species can survive (Hutchinson, 1957; O'Keeffe et al., 2020); for example, for *Borrelia*, the tick-vector and reservoir host (Margos et al., 2019; O'Keeffe et al., 2020). The results here could therefore show that *B. afzelii* and *B. garinii* niche breadth was larger, allowing for colonization of Europe without undergoing a demographic bottleneck and that ecological factors, such as reservoir host associations are instead shaping the patterns of diversity observed in our study. *Borrelia garinii* has indeed been shown to be able to transmit through nonendemic vectors such as *I. scapularis* in laboratory-based studies (Eisen, 2020), potentially highlighting this broad niche in relation to tick vectors. Yet, rigorous vector association studies would be required to fully understand and quantify the niche breadth of the various isolates under study here.

Besides differing in their overall demographic history, each genospecies also showed variable levels of geographic differentiation between and within the two continents. Both *B. afzelii* and *B. bavariensis* showed relatively high F_{ST} values between Asia and Europe whereas *B. garinii* did not (Table 1). This was further supported by AMOVA analyses in which continent explained a large portion of variation along the chromosome for both *B. afzelii* and *B. bavariensis* (Table 2). Previous work based on MLST had hypothesized that these differences in geographic differentiation were due do to *Borrelia* host association and the mobility of host types; with more geographic differentiation expected in less motile hosts (e.g., rodents) and decreased differentiation as hosts become more mobile (e.g., birds) (Gallais et al., 2018; Norte et al., 2020; Vollmer et al., 2011, 2013). The chromosome level analyses reported here

supported these MLST-based studies for the most part with rodent-adapted species (*B. afzelii*, *B. bavariensis*) showing geographic differentiation, while bird-adapted genospecies (*B. garinii*) did not (Tables 1 and 2, Table S7). Even so, we found that within Asia *B. bavariensis* did not show geographic differentiation between sampling sites, which other rodent-associated genospecies (*B. afzelii*) did (Figure 2, Table S7). This pattern was also observed in previous work (Becker et al., 2020), which was unable to directly compare between genospecies as we are able to do here. This brings forward the question, what mechanism(s) could result in this lack of geographic differentiation observed in Asian *B. bavariensis*? One suggestion could be that Asian *B. bavariensis* utilize secondary hosts besides rodents, such as birds, which increase effective dispersal rate. Two *B. bavariensis* isolates (PBi, European; NT29, Asian) were previously shown to be susceptible to complement-active avian sera, which has been taken as a proxy for reservoir host association (Kurtenbach et al., 1998; Kurtenbach et al., 2002). However, as the Asian population is quite diverse (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2013), it is possible that a single isolate would not be representative of the entire population. Recently, *B. bavariensis* DNA was found far afield of its Eurasian range in seabird associated ticks (*I. uriae*) in Canada (Munro et al., 2017) and previous work did indeed suggest that similar genotypes of *B. bavariensis* (described as rodent-adapted *B. garinii*) could spread from mainland Asia to Japan through migratory birds (Ishiguro et al., 2005). Even so, laboratory-based verification of this hypothesized bird-adaptation would be required and cannot be definitively shown solely based on genetic based analyses.

One aspect of our study that warrants consideration is how the *Borrelia* sampling scheme could potentially bias the results presented here: especially considering the inclusion of LB patient isolates. In our data set, European samples predominantly arise from Germany and the Asian samples from Japan (Table S1). By including 17 Russian isolates (*B. bavariensis* & *B. garinii*), we were able to further comment on how differentiation across Asia occurs. Even so, our analysis does not include finer-scale sampling across the Eurasian range which could miss some aspects of how these *Borrelia* genospecies migrated westward from Asia. To the best of our knowledge, the analysis presented here is still the largest genomic level analysis of these three genospecies to date but future work should include fine-scale sampling across Europe and Asia (as shown in Walter et al., 2017 in North America) to clarify further aspects of the westward migration of these genospecies. In addition to this, we include many patient isolates which could bias the results as nonrandom samples. We could test that this was not the case between randomly and non-randomly sampled *B. garinii* isolates (Table S7). Yet, human isolates did seem to differ in their plasmid content, highlighting the importance of using core genomic compartments in our analysis. Even so, all European *B. bavariensis* isolates do come from LB patients and thus the observed bottleneck could be an artefact of sampling only a low diversity subset of European *B. bavariensis* that is capable of human infection. Eight MLST *B. bavariensis* profiles sequenced from *I. ricinus* DNA exist in the PubMLST database (Margos et al., 2008, et al., 2015) and do not differ from patient isolates on these loci, which can roughly proxy the full chromosome diversity (Figure S6). Furthermore, both *B. garinii* ($n = 21$, $\pi_{Human} = 0.005956$) and *B. afzelii* ($n = 11$, $\pi_{Human} = 0.002107$) LB patient isolates included in this study did not display the same reduction in diversity when compared to the full data set (Table 2). This suggests that the bottleneck observed in *B. bavariensis* is genuine as argued previously (Becker et al., 2020; Gatzmann et al., 2015; Margos et al., 2019), although future work should try to include tick-isolates where possible.

The results presented here show how the evolutionary history of each *Borrelia* genospecies is unique and that it is challenging to generalize from one genospecies to the other, further highlighting the need for comparative studies among genospecies to better understand how these bacteria have evolved. The analysis here additionally shows how vector-borne pathogens can display multiple, independent instances of vector and host colonization and how such colonization facilitates the emergence of these pathogens into new environments with potential consequences to both human and animal disease. The results further show that geographic differentiation and potential for migration of vector-borne pathogens, at least in the tick-vectored system, is inherently linked to the reservoir host species. Our results allow for a better understanding of how these two factors (vector- and host-association) differently influence the evolution of vector-borne pathogens and the power of core-genomic analyses in disentangling these factors.

AUTHOR CONTRIBUTIONS

Gabriele Margos, Volker Fingerle, and Noémie S. Becker designed the study concept. Robert E. Rollins, Noémie S. Becker, Mohammed T. Tawfeeq, Fernanda Herrera-Mesías, Hiroki Kawabata, Kozue Sato, and Minoru Nakao collected tick samples and Robert E. Rollins, Noémie S. Becker, Mohammed T. Tawfeeq, Fernanda Herrera-Mesías, Kozue Sato, and Hiroki Kawabata performed *Borrelia* isolations. Robert E. Rollins and Hiroki Kawabata were responsible for morphological identification of all tick specimens. Hiroki Kawabata, Sergey Kovalev, Gabriele Margos, and Volker Fingerle provided additional *Borrelia* isolates and sequence data. Robert E. Rollins sequenced all novel *Borrelia* and assembled all sequence data. Robert E. Rollins ran all analysis with the guidance of Noémie S. Becker and Ricardo J. Pereira. Robert E. Rollins wrote the manuscript with Noémie S. Becker, Ricardo J. Pereira, Hiroki Kawabata, and Gabriele Margos. The final manuscript was read and approved by all coauthors.

ACKNOWLEDGEMENTS

The Pacific Bioscience SMRT sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no), a national technology platform hosted by the University of Oslo and supported by the "Functional Genomics" and "Infrastructure" programmes of the Research Council of Norway and the South-eastern Regional Health Authorities. We would further like to thank Dr Kristian Ullrich for his support in bioinformatic analyses. We would like to thank all past members of the Evolutionary Biology group at

the LMU, all laboratory technicians and colleagues at the National Reference Centre for *Borrelia*, the National Institute of Infection Diseases in Tokyo for allowing us to work in their facilities, all members of the Evolutionary Ecology of Variation group of the Max Plank Institute for Ornithology and the Behavioural Ecology group at LMU for allowing us to collect ticks in their study plots. This work was done in association with the Graduate School of Life Science, Munich. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

The project was funded through the German Research Foundation (DFG grant no. BE 5791/2–1) (NSB and RER). The National Reference Centre for *Borrelia* was funded by the Robert-Koch-Institut, Berlin (VK). This research was further supported by AMED under grant numbers JP20wm0225016 and JP20fk0108068 (HK and KS).

CONFLICT OF INTEREST

The authors have no conflicts of interest to report at the time of publishing.

DATA AVAILABILITY STATEMENT

Full chromosome sequences have been made available through GenBank associated with the BioProjects PRJNA327303, PRJNA449844, and PRJNA722378. The phylogenetic tree, alignments, and R scripts have been deposited to a Dryad Digital repository and can be accessed at https://doi.org/10.5061/dryad.m37pvmd28.

ORCID

Robert E. Rollins https://orcid.org/0000-0002-5779-7001
Ricardo J. Pereira https://orcid.org/0000-0002-8076-4822
Gabriele Margos https://orcid.org/0000-0001-5304-9615

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Arnold, B. J., Huang, I. T., & Hanage, W. P. (2022). Horizontal gene transfer and adaptive evolution in bacteria. *Nature Reviews Microbiology*, *20*(4), 206–218. https://doi.org/10.1038/s41579-021-00650-4

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., … Zagnitko, O. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC Genomic*, *9*(1). https://doi.org/10.1186/1471-2164-9-75

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

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. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

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

Bolger, A. M., Lohse, M., & Usadel, B. (2014a). Genome analysis Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bolger, A. M., Lohse, M., & Usadel, B. (2014b). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., de Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*, 1–9. https://doi.org/10.1186/1471-2105-10-421

Casjens, S., & Huang, W. M. (1993). Linear chromosomal physical and genetic map of *borrelia burgdorferi*, the Lyme disease agent. *Molecular Microbiology*, *8*(5), 967–980. https://doi.org/10.1111/j.1365-2958.1993.tb01641.x

Casjens, S., Mongodin, E. F., Qiu, W. G., Luft, B. J., Schutzer, S. E., Gilcrease, E. B., Huang, W. M., Vujadinovic, M., Aron, J. K., Vargas, L. C., Freeman, S., Radune, D., Weidman, J. F., Dimitrov, G. I., Khouri, H. M., Sosa, J. E., Halpin, R. A., Dunn, J. J., & Fraser, C. M. (2012). Genome stability of Lyme disease spirochetes: Comparative genomics of *Borrelia burgdorferi* plasmids. *PLoS ONE*, *7*(3), e33280. https://doi.org/10.1371/journal.pone.0033280

Charrier, N. P., Hermouet, A., Hervet, C., Agoulon, A., Barker, S. C., Heylen, D., Toty, C., McCoy, K. D., Plantard, O., & Rispe, C. (2019). A transcriptome-based phylogenetic study of hard ticks (Ixodidae). *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-49641-9

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. *Emerging Infectious Diseases*, *12*(7), 1087–1095.

Comstedt, P., Asokliene, L., Eliasson, I., Olsen, B., Wallensten, A., Bunikis, J., & Bergström, S. (2009). Complex population structure of Lyme borreliosis group spirochete *Borrelia garinii* in subarctic Eurasia. *PLoS ONE*, *4*(6), e5841. https://doi.org/10.1371/journal.pone.0005841

Comstedt, P., Jakobsson, T., & Bergström, S. (2011). Global ecology and epidemiology of *Borrelia garinii* spirochetes. *Infection Ecology & Epidemiology*, *1*(1), 9545. https://doi.org/10.3402/iee.v1i0.9545

Csárdi, G., & Nepusz, T. (2006). The igraph software package for complex network research title. *InterJournal*, *Complex Systems*, *1695*(5), 1–9. Retrieved from https://igraph.org

Delcher, A. L., Phillippy, A., Carlton, J., & Salzberg, S. L. (2002). Fast algorithms for large-scale genome alignment and comparison. *Nucleic Acids Research*, *30*(11), 2478–2483. https://doi.org/10.1093/nar/30.11.2478

Drummond, A. J., Ho, S. Y. W., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, *4*(5), 699–710. https://doi.org/10.1371/journal.pbio.0040088

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*(214), 1–8. https://doi.org/10.1186/1471-2148-7-214

Edgar, R. C. (2004a). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, *5*, 113. https://doi.org/10.1186/1471-2105-5-113

Edgar, R. C. (2004b). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Eisen, L. (2020). Vector competence studies with hard ticks and *Borrelia burgdorferi* sensu lato spirochetes: A review. *Ticks and Tick-Borne Diseases*, *11*(3), 101359. https://doi.org/10.1016/j.ttbdis.2019.101359

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data Laurent. *Genetics*, *131*, 479–491. https://doi.org/10.3354/meps198283

Fraser, C., Casjens, S., & Huang, W. (1997). Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature*, *390*(December), 580–586. https://doi.org/10.1038/37551

Gallais, F., De Martino, S. J., Sauleau, E. A., Hansmann, Y., Lipsker, D., Lenormand, C., Talagrand-Reboul, E., Boyer, P. H., Boulanger, N., Jaulhac, B., & Schramm, F. (2018). Multilocus sequence typing of clinical *Borreliella afzelii* strains: Population structure and differential ability to disseminate in humans. *Parasites and Vectors*, *11*(1), 1–13. https://doi.org/10.1186/s13071-018-2938-x

Gatzmann, F., Metzler, D., Krebs, S., Blum, H., Sing, A., Takano, A., Kawabata, H., Fingerle, V., Margos, G., & Becker, N. S. (2015). NGS population genetics analyses reveal divergent evolution of a Lyme Borreliosis agent in Europe and Asia. *Ticks and Tick-Borne Diseases*, *6*(3), 344–351.

Gelman, A., & Su, Y.-S. (2016). Arm: Data analysis using regression and multilevel/hierarchical models. Retrieved from https://cran.r-project.org/package=arm

Haas, B. J., Delcher, A. L., Wortman, J. R., & Salzberg, S. L. (2004). DAGchainer: A tool for mining segmental genome duplications and synteny. *Bioinformatics*, *20*(18), 3643–3646. https://doi.org/10.1093/bioinformatics/bth397

Hoen, A. G., Margos, G., Bent, S. J., Diuk-Wasser, M. A., Barbour, A., Kurtenbach, K., & Fish, D. (2009). Phylogeography of *Borrelia burgdorferi* in the eastern United States reflects multiple independent Lyme disease emergence events. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(35), 15013–15018. https://doi.org/10.1073/pnas.0903810106

Hudson, R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics*, *132*(2), 583–589. https://doi.org/10.1093/genetics/132.2.583

Hudson, R., & Kaplan, N. (1985). Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics*, *111*, 147–164.

Hutchinson, G. E. (1957). Population studies - animal ecology and demography - concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427.

Ishiguro, F., Takada, N., & Masuzawa, T. (2005). Molecular evidence of the dispersal of Lyme disease *Borrelia* from the Asian continent to Japan via migratory birds. *Japanese Journal of Infectious Diseases*, *58*(3), 184–186.

Kamvar, Z. N., Brooks, J. C., & Grünwald, N. J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, *6*, 208. https://doi.org/10.3389/fgene.2015.00208

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kraiczy, P. (2016). Hide and seek: How Lyme disease spirochetes overcome complement attack. *Frontiers in Immunology*, *7*, 385. https://doi.org/10.3389/fimmu.2016.00385

Kurtenbach, K., De Michelis, S., Etti, S., Schäfer, S. M., Sewell, H. S., Brade, V., & Kraiczy, P. (2002). Host association of *Borrelia burgdorferi* sensu lato - The key role of host complement. *Trends in Microbiology*, *10*(2), 74–79.

Kurtenbach, K., Hanincová, K., Tsao, J. I., Margos, G., Fish, D., & Ogden, N. H. (2006). Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nature Reviews Microbiology*, *4*(9), 660–669.

Kurtenbach, K., Sewell, H.-S., Ogden, N. H., Randolph, S. E., & Nuttall, P. A. (1998). Serum complement sensitivity as a key factor in Lyme disease ecology. *Infection and Immunity*, *66*(3), 1248–1251.

Kurtz, S., Phillippy, A., Delcher, A. L., Smoot, M., Shumway, M., Antonescu, C., & Salzberg, S. L. (2004). Versatile and open software for comparing large genomes. *Genome Biology*, *5*, R12 Retrieved from http://www.tigr.org/software/mummer

Lemey, P., Rambaut, A., Drummond, A. J., & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS Computational Biology*, *5*(9), e1000520. https://doi.org/10.1371/journal.pcbi.1000520

Margos, G., Binder, K., Dzaferovic, E., Hizo-Teufel, C., Sing, A., Wildner, M., Fingerle, V., & Jolley, K. A. (2015). PubMLST.org – The new home for the Borrelia MLSA database. *Ticks and Tick-Borne Diseases*, *6*(6), 869–871. https://doi.org/10.1016/j.ttbdis.2015.06.007

Margos, G., Fingerle, V., & Reynolds, S. (2019). *Borrelia bavariensis*: Vector switch, niche invasion, and geographical spread of a tick-borne bacterial parasite. *Frontiers in Ecology and Evolution*, *7*(October), 1–20. https://doi.org/10.3389/fevo.2019.00401

Margos, G., Gatewood, A. G., Aanensen, D. M., Ra Hanincová, K., Terekhova, D., Vollmer, S. A., Cornet, M., Piesman, J., Donaghy, M., Bormane, A., Hurn, M. A., Feil, E. J., Fish, D., Casjens, S., Wormser, G. P., Schwartz, I., & Kurtenbach, K. (2008). MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(25), 8730–8735 Retrieved from www.pnas.org/cgi/content/full/

Margos, G., Vollmer, S. A., Ogden, N. H., & Fish, D. (2011). Population genetics, taxonomy, phylogeny and evolution of *Borrelia burgdorferi* sensu lato. *Infection, Genetics and Evolution*, *11*(7), 1545–1563.

Margos, G., Wilske, B., Sing, A., Hizo-Teufel, C., Cao, W. C., Chu, C., Scholz, H., Straubinger, R. K., & Fingerle, V. (2013). *Borrelia bavariensis* sp. nov. is widely distributed in Europe and Asia. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Part 11), 4284–4288.

Munro, H. J., Ogden, N. H., Lindsay, L. R., Robertson, G. J., Whitney, H., & Lang, S. (2017). Evidence for *Borrelia bavariensis* infections of ixodes uriae within seabird colonies of the North Atlantic Ocean. *Applied and Environmental Microbiology*, *83*(20), 1–9.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: A practical guide for biologists. *Biological Reviews*, *85*(4), 935–956.

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press.

Norte, A. C., Margos, G., Becker, N. S., Albino Ramos, J., Núncio, M. S., Fingerle, V., Araújo, P. M., Adamík, P., Alivizatos, H., Barba, E., Barrientos, R., Cauchard, L., Csörgő, T., Diakou, A., Dingemanse, N. J., Doligez, B., Dubiec, A., Eeva, T., Flaisz, B., & Lopes de Carvalho, I. (2020). Host dispersal shapes the population structure of a tick-borne bacterial pathogen. *Molecular Ecology*, *29*(3), 485–501. https://doi.org/10.1111/mec.15336

O'Keeffe, K. R., Oppler, Z. J., & Brisson, D. (2020). Evolutionary ecology of Lyme *Borrelia*. *Infection, Genetics and Evolution*, *85*(June), 104570. https://doi.org/10.1016/j.meegid.2020.104570

Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A. R., Xia, F., & Stevens, R. (2014). The SEED and the rapid

annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Research*, *42*(D1), D206–D214. https://doi.org/10.1093/nar/gkt1226

Paradis, E. (2010). Pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An efficient swiss army knife for population genomic analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. https://doi.org/10.1093/molbev/msu136

Pollack, R. J., Telford, S. R., & Spielman, A. (1993). Standardization of medium for culturing Lyme disease spirochetes. *Journal of Clinical Microbiology*, *31*(5), 1251–1255. https://doi.org/10.1128/jcm.31.5.1251-1255.1993

Preac-Mursic, V., Wilske, B., & Schierz, G. (1986). European *Borrelia burgdorferi* isolated from humans and ticks culture conditions and antibiotic susceptibility. *Zentralblatt Für Bakteriologie, Mikrobiologie, Und Hygiene*, *263*(1–2), 112–118.

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rollins, R. E., Yeyin, Z., Wyczanska, M., Alig, N., Hepner, S., Fingerle, V., Margos, G., & Becker, N. S. (2021). Spatial variability in prevalence and genospecies distributions of *Borrelia burgdorferi* sensu lato from ixodid ticks collected in southern Germany. *Ticks and Tick-Borne Diseases*, *12*(1), 101589. https://doi.org/10.1016/j.ttbdis.2020.101589

Schwartz, I., Margos, G., Casjens, S. R., Qiu, W. G., & Eggers, C. H. (2021). Multipartite genome of Lyme disease *Borrelia*: Structure, variation and prophages. *Current Issues in Molecular Biology*, *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. (2011). Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clinical Microbiology and Infection*, *17*, 69–79.

Steere, A. C., Strle, F., Wormser, G. P., Hu, L. T., Branda, J. A., Hovius, J. W. R., Li, X., & Mead, P. S. (2016). Lyme borreliosis. *Nature Reviews Disease Primers*, *2*, 16090. https://doi.org/10.1038/nrdp.2016.90

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585–595.

Takano, A., Nakao, M., Masuzawa, T., Takada, N., Yano, Y., Ishiguro, F., Fujita, H., Ito, T., Ma, X., Oikawa, Y., Kawamori, F., Kumagai, T., Hanaoka, N., Ando, S., Honda, N., Taylor, K., Tsubota, T., Konnai, S., & Kawabata, H. (2011). Multilocus sequence typing implicates rodents as the main reservoir host of human-pathogenic *Borrelia garinii* in Japan. *Journal of Clinical Microbiology*, *49*(5), 2035–2039. https://doi.org/10.1128/JCM.02544-10

Takano, A., Toyomane, K., Konnai, S., Ohashi, K., Nakao, M., Ito, T., Andoh, M., Maeda, K., Watarai, M., Sato, K., & Kawabata, H. (2014). Tick surveillance for relapsing fever spirochete *Borrelia miyamotoi* in Hokkaido, Japan. *PLoS ONE*, *9*(8), e104532. https://doi.org/10.1371/journal.pone.0104532

Tavaré, S. (1986). Some probabilistic and statistial problems in the analysis of DNA sequences. *Lectures on Mathematics in Teh Life Sciences*, *17*, 57–86.

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. *Scientific Reports*, *8*(1), 10552. https://doi.org/10.1038/s41598-018-28908-7

Ullrich, K. (2020). CRBHits: From conditional reciprocal best hits to codon alignments and Ka/Ks in R. *Journal of Open Source Software*, *5*(55), 2424. https://doi.org/10.21105/joss.02424

Vollmer, S. A., Bormane, A., Dinnis, R. E., Seelig, F., Dobson, A. D. M., Aanensen, D. M., James, M. C., Donaghy, M., Randolph, S. E., Feil, E. J., Krtenbach, K., & Margos, G. (2011). Host migration impacts on the phylogeography of Lyme Borreliosis spirochaete species in Europe. *Environmental Microbiology*, *13*(1), 184–192. https://doi.org/10.1111/j.1462-2920.2010.02319.x

Vollmer, S. A., Feil, E. J., Chu, C. Y., Raper, S. L., Cao, W. C., Kurtenbach, K., & Margos, G. (2013). Spatial spread and demographic expansion of Lyme borreliosis spirochaetes in Eurasia. *Infection, Genetics and Evolution*, *14*(1), 147–155. https://doi.org/10.1016/j.meegid.2012.11.014

Wallace, R. G., HoDac, H., Lathrop, R. H., & Fitch, W. M. (2007). A statistical phylogeography of influenza A H5N1. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(11), 4473–4478. https://doi.org/10.1073/pnas.0700435104

Walter, K. S., Carpi, G., Caccone, A., & Diuk-Wasser, M. A. (2017). Genomic insights into the ancient spread of Lyme disease across North America. *Nature Ecology and Evolution*, *1*(10), 1569–1576. https://doi.org/10.1038/s41559-017-0282-8

Wolcott, K. A., Margos, G., Fingerle, V., & Becker, N. S. (2021). Host association of *Borrelia burgdorferi* sensu lato: A review. *Ticks and Tick-Borne Diseases*, *12*(June), 101766. https://doi.org/10.1016/j.ttbdis.2021.101766

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rollins, R. E., Sato, K., Nakao, M., Tawfeeq, M. T., Herrera-Mesías, F., Pereira, R. J., Kovalev, S., Margos, G., Fingerle, V., Kawabata, H., & Becker, N. S. (2023). Out of Asia? Expansion of Eurasian Lyme borreliosis causing genospecies display unique evolutionary trajectories. *Molecular Ecology*, *32*, 786–799. https://doi.org/10.1111/mec.16805