Out of Asia? Vector switches leading to the expansion of Eurasian Lyme disease bacteria

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June 12, 2021

Abstract

Vector-borne pathogens exist in obligate transmission cycles between vector and reservoir host species. Host shifts can lead to geographic expansion and the emergence of new diseases. Three etiological agents of human Lyme borreliosis (Borrelia afzelii, Borrelia bavariensis, and Borrelia garinii) predominantly utilize two distinct tick species as vectors in Asia (Ixodes persulcatus) and Europe (Ixodes ricinus) but how and in which order they colonized each continent remains unknown. Here, by reconstructing the evolutionary history of 142 Eurasian isolates, we show that all three Borrelia genospecies evolved from an Asian origin, suggesting that successful expansion into Europe resulted through invading a novel vector. The pattern of gene flow between continents is different between genospecies and most likely conditioned by reservoir host association and their dispersal. Our results highlight that Eurasian Lyme borreliosis agents are all capable of geographic expansion through vector shifts, but potentially differ in their capacity as emergent pathogens.

Introduction

Lyme borreliosis (LB), also termed Lyme disease, is the most common vector-borne disease in the Northern hemisphere (Stanek et al., 2011; Steere, Coburn, & Glickstein, 2004), caused by certain genospecies of*Borrelia* bacteria (Kurtenbach et al., 2006; Gabriele Margos, Fingerle, & Reynolds, 2019; Stanek et al., 2011). These spirochete bacteria are maintained naturally in obligatory transmission cycles between tick vectors and specific vertebrate reservoir hosts (Kurtenbach et al., 2006). In North America human LB is predominantly caused by*Borrelia burgdorferi* sensu stricto (*Bb* ss) while three additional genospecies act as causative agents across Eurasia (*Borrelia afzelii* , *Borrelia bavariensis* , *Borrelia garinii*) (Kurtenbach et al., 2006; Gabriele Margos et al., 2019; Stanek et al., 2011). Genomic analyses have already shown a complex ancestral spread of *Bb* ss across North America (Walter, Carpi, Caccone, & Diuk-Wasser, 2017) which is also observed in specific Eurasian genospecies (Becker et al., 2020; Ana Cláudia Norte et al., 2020). The Eurasian genospecies offer a unique opportunity to understand the geographic expansion of *Borrelia* spirochetes using comparative genomics. However, no study has integrated genomic data from the different genospecies. In particular, no study at the population-level of Asian *B. afzelii* has been published to date.

Borrelia genospecies cannot transmit successfully through all tick species (Eisen, 2020; Gabriele Margos et al., 2019) and can only infect specific vertebrate classes (i.e. rodents, passerines, sea-birds, etc.) while being easily cleared by the immune systems of others (Kurtenbach et al., 2006; Kurtenbach, Sewell, Ogden,

Randolph, & Nuttall, 1998; Gabriele Margos et al., 2019). Eurasian *Borrelia*genospecies currently exist in separate transmission cycles vectored predominately by two generalist tick species in Asia (*Ixodes persulcatus*) and Europe (*Ixodes ricinus*) (Kurtenbach et al., 2006) (Figure 1). This suggests that each genospecies successfully invaded a novel tick vector resulting in the expansion into a new continental transmission cycle. However, how and in which order this expansion occurred is still unknown (Figure 1). For *B. bavariensis,* an Asian origin was already hypothesized as the Asian population displays a higher genetic diversity compared to the almost clonal European population (Becker et al., 2020; Gatzmann et al., 2015; Gabriele Margos et al., 2019). European *B. bavariensis* is thought to have undergone a selective bottleneck while colonizing the European tick vector, *I. ricinus* , resulting in the observed clonal structure (Becker et al., 2020; Gatzmann et al., 2015; Gabriele Margos et al., 2019). Whether or not the other genospecies also underwent this bottleneck has never been studied so far. Both *B. afzelii* and*B. bavariensis* utilize rodents as reservoir hosts (Pär Comstedt, Jakobsson, & Bergström, 2011; Kurtenbach et al., 2006; Gabriele Margos et al., 2009) (Figure 1). In comparison, *B. garinii* is adapted to avian host species (Pär Comstedt et al., 2009, 2011), which includes interconnected terrestrial and marine transmission cycles (Figure 1). This association in *B. garinii* is thought to allow for migration between the European and Asian populations which is not accessible to rodent adapted genospecies. (Figure 1).

Each of these genospecies has successfully established into multiple transmission cycles and offers an opportunity to study how*Borrelia* expanded across Eurasia through comparative genomics. Although, no study to date has integrated genomic data from all three Eurasian-distributed genospecies. Here we report the reconstructed evolutionary history of 142 *B. afzelii* , *B. garinii* , and*B. bavariensis* Eurasian isolates based on full genome sequences including the first Japanese *B. afzelii* genomes sequenced. Our results highlight that these genospecies share an Asian origin with support for migration from an ancestral Asian population vectored by*I. persulcatus* into a novel European vector, *I. ricinus* . 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 and how this could relate to the further expansion of human LB.

Materials & Methods

Isolates used and sequencing

For all information on isolates, including origin and source material refer to Table S1. This study utilized DNA of 136 *Borrelia*isolates coming from three human pathogenic species: *B. afzelii*(n=33), *B. garinii* (n=57), and *B. bavariensis* (n =46). Of these, 52 are novel *Borrelia* isolated from ticks collected either in Japan (n=43) or Germany (n=9) (see Text S1 & Tables S3, S4, S5). Additionally, 55 European isolates (*B. afzelii,* n=11; *B. garinii* , n=25; and *B. bavariensis* , n=19) were provided by the German National Reference Center for *Borrelia* at the Bavarian Food and Health Safety Authority. All isolates, expect one tick isolate, were isolated from humans. DNA for additional Japanese human and tick isolates (n = 12) was provided by the National Institute of Infectious Disease in Tokyo, Japan. Finally, previously sequenced Russian *B. bavariensis* (n=7) (Becker et al., 2020) and DNA from 12 additional Russian *B. garinii* tick isolates were included in the study (see Text S1).

Borrelia isolates were cultured either in inhouse-made MKP (Preac-Mursic, Wilske, & Schierz, 1986) (all European isolates) or inhouse-made BSK-H (Pollack, Telford, & Spielman, 1993) (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, Madison, WI, USA) and from all Japanese and Russian isolates using the Wizard® Genomic DNA Purification Kit (Promega, WI, USA). DNA quality (260/280) and concentration were measured using a NanoDrop® 1000 photometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit® 3.0 fluorometer (Thermo Fisher Scientific, MA, USA), respectively.

For all samples, libraries were produced according to the Nextera XT sample preparation guide (Illumina,

San Diego, CA, USA). Library quality was checked using an Agilent TapeStation 2200 (Agilent, Santa Clara, CA, USA) before being sequenced using an Ilumina MiSeq platform according to standard protocol (Illumina, USA) that produced paired end reads of 250bp.

Chromosome assembly and phylogeny reconstruction

Illumina reads were first trimmed for Illumina MiSeq adapter sequences using Trimmomatic v. 0.38 (Bolger, Lohse, & Usadel, 2014) before being assembled using SPAdes v. 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 Suppl. Met.). 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 v. 3.23 from the package MUMmer (Delcher, Phillippy, Carlton, & Salzberg, 2002; Kurtz et al., 2004). Final chromosomes were produced according to the mapping protocol outlined in Becker et al. (2020) (see Suppl. Met.). 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 assembled chromosomes were aligned using MAFFT v. 7.407 (Katoh, Misawa, Kuma, & Miyata, 2002; Katoh & Standley, 2013). Recombination is known to be low on the *Borrelia* chromosome (Gatzmann et al., 2015) but as recombinant regions could bias the phylogenetic signal, we searched for areas of the chromosome violating the four-gamete condition (Richard R Hudson & Kaplan, 1985) (as described in Gatzmann et al. (2015); see Suppl. Met.). Regions with strong violation of the four-gamete condition were assumed to be recombinant and were removed from the final alignments (final alignment length: 936908bp). Phylogeny reconstruction was done in MrBayes v. 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012) with ploidy set to haploid and a GTR (Tavaré, 1986) substitution model with gamma distributed rate variation. Three independent runs were launched and ran for 5 million generations at which point convergence of parameters was checked with Tracer v. 1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Consensus trees were built using the *sumt* command from MrBayes using a respective burn-in of 25%. Convergence to a single topology in all three independent runs was checked manually in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) which was also used to plot the tree shown in Figure 2. Trees were midpoint rooted on the longest branch, which corresponded to the well-established delineation between*B. afzelii* and the monophyletic group containing *B. bavariensis* and *B. garinii* (Becker et al., 2016; Gabriele Margos, Vollmer, Ogden, & Fish, 2011).

Identification of plasmid content through plasmid partitioning genes

Plasmid content was approximated by the number of plasmid partitioning genes present in each assembly, which have been shown to be unique to specific plasmid types and exist as single copies in *Borrelia* (S. Casjens & Huang, 1993; S. R. Casjens et al., 2012; Fraser, Casjens, & Huang, 1997). Identification of plasmid partitioning genes was performed as outlined in Becker et al. (2020) (see Suppl. Met.). Briefly, we used BLAST v.2.8.1 (Altschul, Gish, Miller, Myers, & Lipman, 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% percent identity. After curation, we defined a plasmid being present if at least one of the partitioning genes was present in the assembled contigs.

Statistical and population genetic analyses

All statistical analysis was performed in R v. 3.6.1 (R Core Team, 2019). Genetic diversity (π) (Nei, 1987) and Tajima's Dtest statistic (Tajima, 1989) were estimated in the package *pegas*(Paradis, 2010). Analysis of molecular variance (AMOVA) (Excoffier, Smouse, & Quattro, 1992) was performed using the package *poppr*(Kamvar, Brooks, & Grünwald, 2015) whereas F_{ST}(Nei, 1987) andD_{XY} (R. R. Hudson, Slatkin, &

Maddison, 1992) were estimated with the package*PopGenome* (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014).

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, Maechler, Bolker, & Walker, 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 & Schielzeth (2010).

Results

For phylogenetic and population genetics analyses, we focused on the linear chromosome as it is a core genomic compartment present in all*Borrelia* and is generally used to reconstruct the evolutionary history between genospecies (Becker et al., 2020; S. R. Casjens et al., 2012; Mongodin et al., 2013). *Borrelia* genomes are highly fragmented and can contain over 20 unique linear and circular plasmids (S. R. Casjens et al., 2012; Fraser et al., 1997) which can be highly plastic even within a single genospecies (Becker et al., 2020; S. R. Casjens et al., 2018; Mongodin et al., 2013). *Borrelia* plasmids contain genes related to host and vector adaptation and absence of certain plasmid types have been linked to reduced infectivity (S. R. Casjens et al., 2018; Dulebohn, Bestor, & Rosa, 2013; Ellis et al., 2013; Embers, Alvarez, Ooms, & Philipp, 2008; Grimm et al., 2004, 2005; Lin, Diuk-Wasser, Stevenson, & Kraiczy, 2020). Therefore, their presence could influence the evolution of these bacteria and should be considered. As each plasmid carries specific, partitioning genes (categorized to PFam32, 49, 50, 57/62) and generally exists in single copies per cell (S. Casjens & Huang, 1993; S. R. Casjens et al., 2012; Fraser et al., 1997), we were able to approximate plasmid content in each isolate by searching for these partitioning genes using BLAST v.2.8.1 (Altschul et al., 1990; Camacho et al., 2009) (algorithm:*blastn*)

In total 142 full chromosome sequences were used for further population genetics analysis, of which 136 were assembled *de novo* from Illumina MiSeq data. Final chromosome length ranged from 825.7 to 906.2 kb (mean: 900.0kb) with chromosome coverages from 8 to 572x (mean: 104.6x) (see Table S1). Plasmid content could only be estimated for the 136 samples for which raw MiSeq data was available. For full isolate information see Table S1.

All genospecies display a probable Asian origin

For both *B. afzelii* and *B. garinii* the oldest node separates a clade containing only Asian isolates from a clade containing isolates from both continents (Figure 2A,C) suggesting that the Asian population is ancestral for both genospecies. *Borrelia bavariensis* displays a deep branching between the two continents, and European isolates are characterized by a low divergence and almost clonal expansion as previously described (Becker et al., 2020; Gatzmann et al., 2015) (Figure 2B). Our original analysis did not include Russian*B. afzelii* isolates. A single Russian *B. afzelii* isolate exists with a full chromosome in GenBank, Tom3107 (Accession Number: NZ_CP009212.1). We re-ran the phylogeny utilizing all *B. afzelii*chromosome sequences including Tom3107 and PBi as an outgroup to root the tree (see Suppl. Met.). Tom3107 was basal to the monophyletic European *B. afzelii* clade (Figure S1) suggesting a stepwise colonization from far-east Asia through Russia into Europe, which was not observed in the other two genospecies.

Higher genetic diversity (π (Nei, 1987)) 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(Tajima, 1989) values (Table 1) as expected for bacteria due to the influence of population expansion (Gatzmann et al., 2015; Tajima, 1989). The European samples always showed more negative values (Table 2), suggesting a more

recent expansion into Europe. *Borrelia bavariensis* displayed the largest difference in Tajima's D and also had the largest absolute divergence value (D_{xy} (R. R. Hudson et al., 1992)) in comparison to the other two genospecies hinting that *B. bavariensis*branching is potentially the oldest and that *B. afzelii* is the youngest with the lowest absolute divergence and difference in Tajima'sD (Table 1).

Our dataset, as with many others, includes non-randomly sampled isolates which could lead to biased estimates of population level statistics (Nei, 1987). As our data set includes randomly sampled isolates as well (see Text S1 and Tables S3, S4, S5) we were able to test for potential sampling biases. Interestingly, we did not observe strong bias in any of these statistics (π , F_{ST} ,D_{XY} , Tajima's D) when calculated on datasets containing random and non-random samples (Text S2).

Each genospecies display unique structuring

Borrelia bavariensis displayed the strongest geographic structuring between the European and Asian samples (F_{ST} (Nei, 1987) = 0.744;$AMOVA_{continent}$ (Excoffier et al., 1992) = 69.7% of molecular variance (σ)) followed by *B. afzelii*(F_{ST} = 0.570;$AMOVA_{continent}$ = 40.2% of σ) (Table 1 & 2). Regions (defined as country or sampling locality if known, see Table S1 & S3) within continents further explained variation in *B. afzelii* samples ($AMOVA_{Region}$ = 23.6% of $\sigma\cdot$ Table 2) and structuring was observed between randomly sampled *B. afzelii* isolates from the islands of Hokkaido (ASA) and Honshu (NAG) (F_{ST} = 0.379; Table S2). Honshu and Hokkaido*B. afzelii* isolates do form two reciprocally monophyletic clades, with the notable exception of one Hokkaido isolate belonging to the Honshu clade (Figure 2A) suggesting some level of migration. Of interest however, this trend was not observed for *B. bavariensis*($AMOVA_{Region}$ = 0.99% of $\sigma\cdot$ Table 2) and, indeed, randomly sampled *B. bavariensis* isolates from the islands of Hokkaido and Honshu did not show geographic structuring (F_{ST} = 0.057; Table S2) even though both *B. bavariensis* and *B. afzelii* are rodent adapted (Kurtenbach et al., 2006; Gabriele Margos et al., 2019, 2011). Furthermore, Asian*B. bavariensis* displayed a low divergence clade containing samples from Japan (including isolates from distinct islands), China, and Russia (Figure 1B) suggestive of relatively high migration between Asian regions. Less geographic structuring by continent was observed in*B. garinii* (F_{ST} = 0.13;$AMOVA_{Continent}$ = 8.7% of $\sigma\cdot$ Table 1 & 2) as expected as *B. garinii* displayed little geographic structuring throughout the phylogeny with mixing of samples from different geographic origins (Figure 2C).

Plasmid content is generally homogenous between genospecies

Borrelia afzelii and *B. bavariensis* both differed significantly in plasmid numbers between Europe and Asia, with European*B. afzelii* (two-sided unpaired t-test, $p = 0.03$) and Asian*B. bavariensis* ($p < 0.001$) having significantly more plasmids in comparison to the other geographic population (Figure 3A). *Borrelia garinii* isolates did not differ in overall plasmid number between Asia and Europe ($p = 0.08$) but had significantly fewer plasmids in comparison to both *B. afzelii* populations (Asian, $p = 0.003$; European, $p < 0.001$) and to Asian *B. bavariensis* ($p < 0.001$) (Figure 3A). When we look at the absolute plasmid number for a population, defined as the number of unique plasmid types present in at least one isolate from the population, only European *B. bavariensis* showed a lower absolute plasmid number (black circle; Figure 3A) in comparison to the other species such as *B. garinii* . *Borrelia garinii* also had on average lower plasmid numbers per isolate (comparable to European*B. bavariensis*), but the absolute number of plasmid types present in the population (i.e. diversity of plasmid types) is comparable to*B. afzelii* and Asian *B. bavariensis* (Figure 3A).

Based on the plasmid presence/absence matrix for all samples (n = 136), we further ran a multi-dimensional scaling (MDS) analysis to test if plasmid content corresponds to factors such as continent (i.e. vector) or genospecies. Plasmid content appears more homogenous between Asian isolates (Figure 3B) versus European isolates, which display clusters based on genospecies (Figure 3B). This could result from European isolates representing a subset of available plasmid combinations which are all present in the Asian populations. Even so, no plasmid types were more frequently associated with factors such as genospecies or geography (Figure S2). It is important however to note, as *Borrelia* can lose plasmids due to long-term culturing (G. Margos et al., 2017). Many human isolates (Table S1) have been potentially kept in culture longer suggesting that the current plasmid results could be biased even though sequencing focused on low passage isolates (<10

passages). Tick isolates did indeed have on average higher plasmid content (Table S6; mean: 1.19; 95% CI: 0.16, 2.22) suggesting that this bias may be present for our human isolates.

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 the current major etiological agents of human Lyme borreliosis (LB) in Eurasia (*B. afzelii* ,*B. bavariensis* , and *B. garinii*) are vectored mainly by two different tick species: *I. persulcatus* in Asia, and *I. ricinus* in Europe. This means that each of these genospecies has, at least once during its evolution, successfully invaded a novel tick species and consequently a local population of vertebrate hosts. Yet how this invasion occurred and in which order is currently not known due to a lack of data. Here we report a reconstructed phylogeny of 142 Eurasian isolates belonging to the genospecies *B. afzelii* , *B. bavariensis* , and *B. garinii* . All three genospecies appear to share an Asian origin, suggesting a repeated expansion into Europe in relation to successfully invading a novel tick vector, *I. ricinus* . However, all three genospecies display unique sub-structuring which could be linked to ecological variability in their specific reservoir hosts. The results further show that the observed bottleneck in European *B. bavariensis* isolates argued to be in connection to invading *I. ricinus* (Becker et al., 2020; Gatzmann et al., 2015; Gabriele Margos et al., 2019), is not shared by the other two genospecies. This all suggests that Eurasian Lyme borreliosis agents were all capable of geographic expansion through vector shifts but differ in their capacity as emergent pathogens in relation to potential, future expansions into novel transmission cycles.

Borrelia bavariensis was already argued to have an Asian origin due to the deep branching observed between European and Asian isolates and that the majority of diversity exists in the Asian population (Becker et al., 2020; Gatzmann et al., 2015; Gabriele Margos et al., 2019). This finding is supported by the expanded analysis reported here. One point that warrants consideration is that all European *B. bavariensis* isolates come from humans and that the observed pattern in diversity could potentially be an artifact of sampling only a low diversity sub-set of European *B. bavariensis* better adapted to human infection. As our study includes European human isolates for two additional genospecies, we were able to disprove that this pattern is due to sampling bias through showing that human *B. garinii* or*B. afzelii* isolates do not display a similar reduction in genetic diversity (Text S2). In addition to this, a search of the*Borrelia* MLST (multiple locus sequencing typing) database (Gabriele Margos et al., 2008) shows eight *B. bavariensis* samples coming from *I. ricinus* DNA which do not differ from patient isolates on the eight MLST loci, which can roughly proxy the full chromosome diversity (Figure S3). These data support that the observed pattern in *B. bavariensis* is genuine. Compared to *B. bavariensis* , no research has focused on the geographic origin of*B. afzelii* or *B. garinii* . Previous work raised the hypothesis of an Asian origin for *B. afzelii* but based on very few samples (Takano et al., 2011), whereas for *B. garinii* only partial structuring between continents was previously reported (Ana Cláudia Norte et al., 2020). Here though, we show that both *B. afzelii* and *B. garinii* are characterized by a basal node which splits a fully Asian clade from a clade of mixed geographic origin, suggesting for the first time that all three of these pathogenic genospecies originate in Asia and that through successful colonization of *I. ricinus* were able to expand into Europe. MDS clustering based on plasmid profiles further supported this by suggesting that the plasmid profiles present in Europe are a subset of available profiles present in Asia (Figure 3A). This could further show that the European population stems from the Asian population. *Borrelia afzelii* was the only genospecies which showed a step-wise colonization pattern from far-east Asia through Russia and into Europe (Figure S1) which has been observed in other tick-borne pathogens (Kovalev & Mukhacheva, 2014) suggesting differences in migratory patterns between species. We further hypothesized about which genospecies colonized Europe first through calculating absolute divergence (D_{XY}) and Tajima's D (Tajima, 1989). *Borrelia bavariensis* shows the highest D_{XY} suggesting that this colonization is the oldest of the three with *B. afzelii* then the youngest with the lowest value of D_{XY} . Additionally, as expected from bacterial populations (Gatzmann et al., 2015; Tajima, 1989), Tajima's D values are consistent with population expansion (negative Tajima's D) but the European expansion for each species is younger (more negative values). The magnitude of difference in Tajima's D mirrors that of D_{XY} with*B. bavariensis* showing the lowest difference in Tajima's D(less recent) and *B. afzelii* showing the

highest difference (most recent).

It is apparent from our analysis that, after the colonization of Europe, each genospecies experienced variable levels of gene flow which we argue can be related back to their host associations. The fact that *B. garinii* showed little to no geographic structuring is in accordance with previous results (Pär Comstedt et al., 2009, 2011; Ana Cláudia Norte et al., 2020). *Borrelia garinii* utilizes birds as reservoir hosts and exists in overlapping terrestrial and marine transmission cycles, where it is vectored by different tick species (terrestrial:*I. ricinus* and *I. persulcatus* ; marine: *I. uriae*) (Pär Comstedt et al., 2006, 2011; Gómez-Díaz et al., 2011; A. C. Norte, Ramos, Gern, Núncio, & Lopes de Carvalho, 2013) (Figure 1). The lack of geographic structure observed in *B. garinii* is thought to be a result of this, as birds could aid in the migration of this genospecies (Pär Comstedt et al., 2009, 2011; Ana Cláudia Norte et al., 2020). This would explain why we cannot differentiate between distinct geographic locations. This pattern for *B. garinii* was already observed on a European level (Ana Cláudia Norte et al., 2020), but we are now able to show that it occurs over the whole distribution range of the genospecies. *Borrelia afzelii* and *B. bavariensis*displayed structured populations in our analysis. Within-continent structuring for European *B. afzelii* was previously attributed to utilizing rodents as reservoir hosts (Gallais et al., 2018; Vollmer et al., 2011), which we now propose to also occur in Asian *B. afzelii* populations. Even though our analysis does show some level of migration is possible along the geographic scale of this project as one Hokkaido isolate does cluster within the Honshu clade (Figure 2). As*B. bavariensis* also associates with rodents (Gabriele Margos et al., 2009, 2013), we would expect to also observe geographic structuring. As previously reported, there does not appear to be gene flow between the European and Asian populations suggesting genetic isolation (high F_{ST} andD_{XY} ; Table 1), but within Asia *B. bavariensis* is not structured as expected for a rodent adapted genospecies (Gabriele Margos et al., 2009, 2013). Our analysis builds upon previous work which observed migration between Asian regions (i.e. Japan, China, Russia) (Becker et al., 2020), but by further adding randomly sampled isolates from distinct Japanese islands: Honshu and Hokkaido. Unlike *B. afzelii* , where we observe lower migration between the islands (F_{ST} =0.379; Table S2),*B. bavariensis* isolates do not seem to have the same barrier to migration (F_{ST} =0.057; Table S2). This brings forward the question, what mechanism(s) could result in this unexpected migration of Asian *B. bavariensis* isolates? One suggestion could be that Asian *B. bavariensis* utilize secondary hosts besides rodents which increase effective dispersal rate. Recently, *B. bavariensis* DNA was found far afield of its Eurasian range in seabird associated ticks (*I. uriae*) in Canada (Munro et al., 2017). As there are similarities in the structuring of Asian *B. bavariensis*to *B. garinii* from our results (low F_{ST} , high π , AMOVA with low σ due to geography; Table 1 & 2), it could be that in rare cases *B. bavariensis* may successfully transmit through avian hosts although rodent adapted. This fact had been previously observed where rodent-associated genospecies (i.e. *B. afzelii*) appeared to transmit through avian hosts in Europe (Heylen, Matthysen, Fonville, & Sprong, 2014). Although the extent of transmission appears to be different between *B. bavariensis* and*B. afzelii* based on our analyses. Until 2009 (Gabriele Margos et al., 2009), *B. bavariensis* was considered a sub-type of *B. garinii* which utilized rodents as reservoir hosts (Masuzawa, 2004; Takano et al., 2011). This association with rodents was experimentally shown for two isolates (PBi, European; NT29, Asian) where they were exposed to rodent or avian immune sera and were susceptible to avian sera only (Kurtenbach et al., 2002, 1998). In this case, as in many studies, immune serum resistance is taken as a proxy of reservoir host associations (Kurtenbach et al., 2002, 1998). This result was used to support that *B. bavariensis* is not able to transmit through avian hosts. As the Asian population is quite diverse (Becker et al., 2020; Gatzmann et al., 2015) it is possible that a single isolate will not be representative for the entire population. Previous work did indeed suggest that similar genotypes of *B. bavariensis* (described as rodent adapted *B. garinii*) which were isolated from infected mice in Japan shared unique sequence components to a bird isolated strain from the Korean Peninsula, suggesting that *B. bavariensis* could spread from mainland Asia to Japan through migratory birds (Ishiguro, Takada, & Masuzawa, 2005), as we are proposing. Additionally, a study based on restriction fragment length polymorphism (RFLP) analysis described a novel RFLP type (type IVa) (Nakao, Miyamoto, & Fukunaga, 1994) which is now known to belong to *B. bavariensis* (Dr. Minoru Nakao & Dr. Hiroki Kawabata, personal communication). The isolates belonging to this RFLP type were isolated from rodents, humans, but also birds (Nakao et al., 1994). Whole genome sequencing of these isolates would allow us to confirm if these bird isolates truly belong to*B.*

bavariensis .

The results presented here suggest some answers to how LB spirochetes (*B. afzelii* , *B. bavariensis* , and *B. garinii*) expanded across Eurasia, through showing that all currently known pathogenic Eurasian *Borrelia* genospecies expanded into Europe from an ancestral Asian population through successful colonizing a novel tick vector (*I. ricinus*). Recently, *B. garinii* was found in *I. uriae* ticks in seabird colonies along the Atlantic coast of North America (Pär Comstedt et al., 2009, 2011; Smith et al., 2006). As*B. garinii* was shown to be rarely transmitted through the North American tick vector (*I. scapularis*) in lab based studies (Eisen, 2020) and here we show that *B. garinii* expanded into Europe through colonization of *I. ricinus* , potentially another expansion into the North American transmission cycle is possible if other requirements, such as reservoir host availability, are met. Outside of this, we further observed that post-colonization gene flow appears to relate to host association and were able to make further testable hypotheses regarding the ecology of the populations. Our analysis provides novel information to the spread of LB-causing spirochetes across Eurasia with applications to how adaptation to novel vector species can facilitate geographic expansion and thus potentially aid in the spread of emergent human pathogens.

Acknowledgments

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" programs of the Research Council of Norway and the South-eastern Regional Health Authorities.

We would like to thank all past members of the Evolutionary Biology group at the LMU, all lab 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.

Authors' Contributions

GM, VF, NSB designed the study concept. RER, NSB, MTT, FHM, HK, KS, and MN collected tick samples and RER, NSB, MTT, FHM, KS, and HK performed*Borrelia* isolations. RER and HK were responsible for morphological identification of all tick specimens. HK, SK, GM, and VF provided additional *Borrelia* isolates and sequence data. RER sequenced all novel *Borrelia* and assembled all sequence data. RER ran all analysis with the guidance of NSB and RJP. RER wrote the manuscript with NSB, RJP, HK, and GM. The final manuscript was read and approved by all co-authors.

Data availability

All sequence data are available through GenBank associated with the BioProjects PRJNA327303, PRJNA449844, and PRJNA722378. The phylogenetic tree, alignments, and R scripts used in analysis are currently in the process of being submitted to a Dryad Digital repository. All data and scripts will be released upon publication of the manuscript.

Conflicts of interest

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

Funding

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).

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. doi: 10.1016/S0022-2836(05)80360-2

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., ... 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. doi: 10.1089/cmb.2012.0021

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* , *67* (1–48).

Becker, N. S., Margos, G., Blum, H., Krebs, S., Graf, A., Lane, R. S., ... Fingerle, V. (2016). Recurrent evolution of host and vector association in bacteria of the *Borrelia burgdorferi* sensu lato species complex. *BMC Genomics* , *17* (1), 734–746. Retrieved from http://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-3016-4

Becker, N. S., Rollins, R. E., Nosenko, K., Paulus, A., Martin, S., Krebs, S., ... Margos, G. (2020). High conservation combined with high plasticity: Genomics and evolution of *Borrelia bavariensis* .*BMC Genomics* , *21* , 702. doi: 10.1186/s12864-020-07054-3

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

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. doi: 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. doi: 10.1111/j.1365-2958.1993.tb01641.x

Casjens, S. R., Di, L., Akther, S., Mongodin, E. F., Luft, B. J., Schutzer, S. E., ... Qiu, W. G. (2018). Primordial origin and diversification of plasmids in Lyme disease agent bacteria. *BMC Genomics* , *19* (1), 1–24. doi: 10.1186/s12864-018-4597-x

Casjens, S. R., Mongodin, E. F., Qiu, W. G., Luft, B. J., Schutzer, S. E., Gilcrease, E. B., ... Fraser, C. M. (2012). Genome stability of Lyme disease spirochetes: Comparative genomics of Borrelia burgdorferi plasmids. *PLoS ONE* , *7* (3). doi: 10.1371/journal.pone.0033280

Comstedt, P, Bergstrom, S., Olsen, B., Garpmo, U., Marjavaara, L., Mejlon, H., ... Bunikis, J. (2006). Migratory passerine birds as reserviors of Lyme borreliosis in Europe. *Emerging Infectious Diseases* , *12* (7), 1087–1095.

Comstedt, Pär, 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). doi: 10.1371/journal.pone.0005841

Comstedt, Pär, Bergström, S., Olsen, B., Garpmo, U., Marjavaara, L., Mejlon, H., ... Bunikis, J. (2006). Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. *Emerging Infectious Diseases* , *12* (7), 1087–1095.

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

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. doi: 10.1093/nar/30.11.2478

Dulebohn, D. P., Bestor, A., & Rosa, P. A. (2013). *Borrelia burgdorferi* linear plasmid 28-3 confers a selective advantage in an experimental mouse-tick infection model. *Infection and Immunity* ,*81* (8), 2986–2996. doi: 10.1128/IAI.00219-13

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. doi: 10.1016/j.ttbdis.2019.101359

Ellis, T. C., Jain, S., Linowski, A. K., Rike, K., Bestor, A., Rosa, P. A., ... Jewett, M. W. (2013). In Vivo Expression Technology Identifies a Novel Virulence Factor Critical for *Borrelia burgdorferi* Persistence in Mice. *PLoS Pathogens* , *9* (8). doi: 10.1371/journal.ppat.1003567

Embers, M. E., Alvarez, X., Ooms, T., & Philipp, M. T. (2008). The failure of immune response evasion by linear plasmid 28-1-deficient*Borrelia burgdorferi* is attributable to persistent expression of an outer surface protein. *Infection and Immunity* , *76* (9), 3984–3991. doi: 10.1128/IAI.00387-08

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. doi: 10.3354/meps198283

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

Gallais, F., De Martino, S. J., Sauleau, E. A., Hansmann, Y., Lipsker, D., Lenormand, C., ... 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. doi: 10.1186/s13071-018-2938-x

Gatzmann, F., Metzler, D., Krebs, S., Blum, H., Sing, A., Takano, A., ... 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

Gómez-Díaz, E., Boulinier, 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.*Environmental Microbiology* , *13* (9), 2453–2467. doi: 10.1111/j.1462-2920.2011.02515.x

Grimm, D., Eggers, C. H., Caimano, M. J., Tilly, K., Stewart, P. E., Elias, A. F., ... Rosa, P. A. (2004). Experimental assessment of the roles of linear plasmids lp25 and lp28-1 of *Borrelia burgdorferi* throughout the infectious cycle. *Infection and Immunity* , *72* (10), 5938–5946. doi: 10.1128/IAI.72.10.5938-5946.2004

Grimm, D., Tilly, K., Bueschel, D. M., Fisher, M. A., Policastro, P. F., Gherardini, F. C., ... Rosa, P. A. (2005). Defining plasmids required by *Borrelia burgdorferi* for colonization of tick vector*Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology* , *42* (4), 676–684. doi: 10.1093/jmedent/42.4.676

Heylen, D., Matthysen, E., Fonville, M., & Sprong, H. (2014). Songbirds as general transmitters but selective amplifiers of *Borrelia burgdorferi* sensu lato genotypes in *Ixodes rinicus* ticks.*Environmental Microbiology* , *16* (9), 2859–2868. doi: 10.1111/1462-2920.12304

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

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

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* , *17* (8), 754–755. doi: 10.1093/bioinformatics/17.8.754

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. doi: 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. doi: 10.1093/molbev/mst010

Kovalev, S. Y., & Mukhacheva, T. A. (2014). Tick-borne encephalitis virus subtypes emerged through rapid vector switches rather than gradual evolution. *Ecology and Evolution* , *4* (22), 4307–4316. doi: 10.1002/ece3.1301

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* , R 12. Retrieved from http://www.tigr.org/software/mummer.

Lin, Y. P., Diuk-Wasser, M. A., Stevenson, B., & Kraiczy, P. (2020). Complement Evasion Contributes to Lyme Borreliae–Host Associations.*Trends in Parasitology* , 1–12.

Margos, G., Hepner, S., Mang, C., Marosevic, D., Reynolds, S. E., Krebs, S., ... Fingerle, V. (2017). Lost in plasmids: next generation sequencing and the complex genome of the tick-borne pathogen*Borrelia burgdorferi* . *BMC Genomics* , *18* (1), 422.

Margos, Gabriele, 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. doi: 10.3389/fevo.2019.00401

Margos, Gabriele, Gatewood, A. G., Aanensen, D. M., Hanincová, K., Terekhova, D., Vollmer, S. A., ... 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, Gabriele, Vollmer, S. A., Cornet, M., Garnier, M., Fingerle, V., Wilske, B., ... Kurtenbach, K. (2009). A new *Borrelia*species defined by multilocus sequence analysis of housekeeping genes.*Applied and Environmental Microbiology* , *75* (16), 5410–5416.

Margos, Gabriele, 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, Gabriele, Wilske, B., Sing, A., Hizo-Teufel, C., Cao, W. C., Chu, C., ... 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.

Masuzawa, T. (2004). Terrestrial distribution of the Lyme borreliosis agent *Borrelia burgdorferi* sensu lato in East Asia.*Japanese Journal of Infectious Diseases* , *57* (6), 229–235.

Mongodin, E. F., Casjens, S. R., Bruno, J. F., Xu, Y., Drabek, E. F., Riley, D. R., ... Luft, B. J. (2013). Inter- and intra-specific pan-genomes of *Borrelia burgdorferi* sensu lato: Genome stability and adaptive radiation.

BMC Genomics , *14* (1). doi: 10.1186/1471-2164-14-693

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* . *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.

Nakao, M., Miyamoto, K., & Fukunaga, M. (1994). Lyme Disease Spirochetes in Japan: Enzootic Transmission Cycles in Birds, Rodents, and *Ixodes persulcatus* Ticks. *The Journal of Infectious Diseases* , *170* , 878–882.

Nei, M. (1987). *Molecular Evolutionary Genetics* . New York: Columbia University Press.

Norte, A. C., Ramos, J. A., Gern, L., Núncio, 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. *Environmental Microbiology* , *15* (2), 386–397.

Norte, Ana Cláudia, Margos, G., Becker, N. S., Albino Ramos, J., Núncio, M. S., Fingerle, V., … Lopes de Carvalho, I. (2020). Host dispersal shapes the population structure of a tick-borne bacterial pathogen. *Molecular Ecology* , *29* (3), 485–501. doi: 10.1111/mec.15336

Paradis, E. (2010). Pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics* , *26* (3), 419–420. doi: 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. doi: 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. doi: 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.* Vienna, Austria: 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. doi: 10.1093/sysbio/syy032

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* , *61* (3), 539–542. doi: 10.1093/sysbio/sys029

Smith, R. P., Muzaffar, S. Bin, Lavers, J., Lacombe, E. H., Cahill, B. K., Lubelczyk, C. B., … Rand, P. W. (2006). *Borrelia garinii* in seabird ticks (*Ixodes uriae*), Atlantic Coast, North America. *Emerging Infectious Diseases* , *12* (12), 1909–1912. doi: 10.3201/eid1212.060448

Stanek, G., Fingerle, V., Hunfeld, K.-P., Jaulhac, B., Kaiser, R., Krause, A., … Gray, J. (2011). Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* , *17* , 69–79.

Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *Journal of Clinical Investigation* , *113* (8), 1093–1101. doi: 10.1172/JCI21681

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., ... 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. doi: 10.1128/JCM.02544-10

Tavaré, S. (1986). Some Probabilistic and Statistial Problems in the Analysis of DNA Sequences. *Lectures on Mathematics in Teh Life Sciences* , *17* , 57–86.

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

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. doi: 10.1038/s41559-017-0282-8

Tables and Figure Legends:

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 non-randomly sampled isolates (both tick and human), but values calculated for randomly sampled isolates showed similar statistics (see Text S2 & Table S2).

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[38] 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

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. bavarien-*

sis) or birds (*B. garinii*) as reservoir hosts (Kurtenbach et al., 2006; Gabriele Margos et al., 2019, 2011). *Borrelia garinii* specifically utilizes interconnected terrestrial and marine based transmission cycles (P Comstedt et al., 2006; Pär Comstedt et al., 2009, 2011). In marine systems, this species is maintained by seabird reservoir host species and the vector *I. uriae* (Pär 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 disease (Kurtenbach et al., 2006; Stanek et al., 2011).

Figure 2. Phylogeny of *B. afzelii* , *B. bavariensis* , and *B. garinii* based on the main chromosome corrected for recombining regions (see Suppl. Met.). The phylogeny was reconstructed with MrBayes v. 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012) with ploidy set to haploid and a GTR (Tavaré, 1986) substitution model with gamma distributed rate variation. Three independent runs were launched and ran for 5 million generations each at which point convergence of parameters was checked with Tracer v. 1.7.1 (Rambaut et al., 2018). Consensus trees were built using the *sumt*command from MrBayes using a respective burn-in of 25%. The collapsed tree displays the full phylogeny (where monophyletic groups are collapsed if all isolates come from the same geographic origin) and then the expanded tree is shown independently for *B. afzelii* (A),*B. bavariensis* (B), and *B. garinii* (C). Colors correspond to geographic origin of the isolates: Europe (blue), Japan (red), purple (Russia), orange (China). For Japanese tick isolates, the island of origin is shown either as a diamond (Hokkaido) or star (Honshu) when known. The scale bar is in substitutions per site.

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).