

Evolutionary Genetics of *Borrelia*

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Abstract

The genus *Borrelia* consists of evolutionarily and genetically diverse bacterial species that cause a variety of diseases in humans and domestic animals. These vector-borne spirochetes can be classified into two major evolutionary groups, the Lyme borreliosis clade and the relapsing fever clade, both of which have complex transmission cycles during which they interact with multiple host species and arthropod vectors. Molecular, ecological, and evolutionary studies have each provided significant contributions towards our understanding of the natural history, biology and evolutionary genetics of *Borrelia* species; however, integration of these studies is required to identify the evolutionary causes and consequences of the genetic variation within and among *Borrelia* species. For example, molecular and genetic studies have identified the adaptations that maximize fitness components throughout the *Borrelia* lifecycle and enhance transmission efficacy but provide limited insights into the evolutionary pressures that have produced them. Ecological studies can identify interactions between *Borrelia* species and the vertebrate hosts and arthropod vectors they encounter and the resulting impact on the geographic distribution and abundance of spirochetes but not the genetic or molecular basis underlying these interactions. In this review we discuss recent findings on the evolutionary genetics from both of the evolutionarily distinct clades of *Borrelia* species. We focus on connecting molecular interactions to the ecological processes that have driven the evolution and diversification of *Borrelia* species in order to understand the current distribution of genetic and molecular variation within and between *Borrelia* species.

Introduction

Zoonotic pathogens, like those that comprise the *Borrelia* genus, are a major cause of emerging and re-emerging infectious diseases worldwide and constitute a significant threat to public health (Jones et al., 1994; Taylor et al., 2001). Like many of the bacterial and viral species that cause zoonotic diseases, most *Borrelia* species have complex transmission cycles in which they interact with multiple vertebrate hosts and vectors over the course of their life cycles (Taylor et al., 2001; Lloyd-Smith et al., 2009; Huang et al., 2019). The bacterial spirochetes belonging to the genus *Borrelia* are vectored by arthropods and infect a wide range of vertebrate hosts; many cause diseases in humans and domestic animals (Burgdorfer et al., 1982; Parker and White, 1992; Pritt et al., 2016), please see Radolf and Samuels (2021). Species within the genus *Borrelia* are classified into two major evolutionary groups, the Lyme borreliosis clade (LB) and the relapsing fever clade (RF). Members of the Lyme borreliosis clade are vectored exclusively by hard-bodied ticks in the *Ixodes* genus and have been investigated to a much greater depth than members of the relapsing fever clade, most of which are vectored by soft-bodied *Argasid* ticks. Although these divergent evolutionary clades recently have been proposed as separate genera (Adeolu and Gupta, 2014), we will continue to refer to them as the Lyme borreliosis clade and the relapsing fever clade until the new classifications have additional consensus (Adeolu and Gupta, 2014; Barbour et al., 2017; Margos et al., 2017, 2020). In this article, we review the ecological interactions underlying the evolutionary forces that have produced the current distribution of genetic and genomic variation in both the LB and RF *Borrelia* clades (Table 1). In addition, we focus on the evolutionary and ecological

Table 1

		All <i>Borrelia</i>	Lyme <i>Borrelia</i>	RF <i>Borrelia</i>
Vector	Vector specialization	<ul style="list-style-type: none"> • Geographic range shaped by vector associations (Margos et al., 2011, 2019a) • Vector switch can result in genetic divergence among subpopulations, expansion into novel geographic range (Lescot et al., 2008; Gatzmann et al., 2015; Becker et al., 2016) 	<ul style="list-style-type: none"> • Characterized molecular factors contributing to tick specialization and persistence (e.g. ospA) (Pal et al., 2004; Battisti et al., 2008; Konnai et al., 2012) • Long feeding time of <i>Ixodid</i> ticks provides opportunities for migration and gene flow (Sonenshine, 1979; Margos et al., 2011; Vollmer et al., 2011b; Norte et al., 2020b) • Rate and direction of gene flow only weakly correlated with that of ticks (Walter et al., 2017) 	<ul style="list-style-type: none"> • Transovarial transmission in some species, although molecular mechanisms poorly characterized (Barbour and Hayes, 1986; Cutler, 2010; Rollend et al., 2013) • Short feeding time of <i>Argasid</i> ticks limits opportunities for migration and gene flow (Vial et al., 2006; Vial, 2009)
	Life inside vector environment	<ul style="list-style-type: none"> • Exposure to environmental conditions experienced by vectors (Kung et al., 2013) 	<ul style="list-style-type: none"> • Many genes regulated in temperature-dependent manner likely due to exposure to fluctuating environmental conditions in hard <i>Ixodid</i> ticks (Ojaimi et al., 2003; Tokarz et al., 2004; Popitsch et al., 2017; Phelan et al., 2019) 	<ul style="list-style-type: none"> • Sheltered microhabitats of <i>Argasid</i> ticks allow spirochetes to survive and remain infectious for several years (Francis, 1938)
	Microbial interactions within the vector	<ul style="list-style-type: none"> • Limited number of genes involved in interbacterial interaction pathways owing to limited density and diversity of resident microbial communities in ticks (Ross et al., 2018; Couper et al., 2019) 	<ul style="list-style-type: none"> • Frequent coinfection with other human pathogens (Hersh et al., 2014; Diuk-Wasser et al., 2016; Moutailler et al., 2016; Ross et al., 2018) • Competition with coinfecting strains reduces spirochete load in ticks (Rego et al., 2014; Moutailler et al., 2016; Walter et al., 2016; Durand et al., 2017; Genne et al., 2018; Genné et al., 2019) 	<ul style="list-style-type: none"> • <i>Limited data have been collected on coinfections of RF Borrelia with conspecific strains or other human pathogens</i>
Host	Host specialization	<ul style="list-style-type: none"> • <i>Limited data have been collected on host associations within RF species, so it is unknown if the molecular mechanisms or evolutionary genetic impacts found among LB species apply to RF Borrelia species</i> 	<ul style="list-style-type: none"> • Well characterized genetic and molecular mechanisms contributing to host specialization (e.g. CspA, OspC, OspE, etc.) (Marconi et al., 1993; Livey et al., 1995; Lagal et al., 2006; Onder et al., 2012; Hammerschmidt et al., 2014b; Hart et al., 2018; Tufts et al., 2019; Lin et al., 2020) • Limited HGT among species with different host associations (Jacquot et al., 2014) • Host associations shape population genetic structure (e.g. bird-associated species have much less genetic structure than rodent-associated species) (Victorino et al., 2008; Vollmer et al., 2011b, 2013; Šimo et al., 2017; Norte et al., 2020b) • Host switch results in genetic divergence among subpopulations, expansion into novel geographic range (Margos et al., 2009; Becker et al., 2016) • Wide geographic range of vectors permits hosts to drive changes in geographic range (Stanek et al., 2012; Šimo et al., 2017) • Diverse host communities maintain genetic diversity of <i>Borrelia</i> populations (Brisson and Dykhuizen, 2004; Mechai et al., 2016; Vuong et al., 2017) 	<ul style="list-style-type: none"> • Limited geographic range of vectors limits extent to which hosts can drive changes in geographic range (Vial, 2009; Trape et al., 2013)
	Interactions with host immune system	<ul style="list-style-type: none"> • Host immune memory drives negative frequency dependent selection on <i>Borrelia</i> populations (Marcsisin et al., 2016; Gomez-Chamorro et al., 2019) • Well characterized genetic and molecular mechanisms that enable rapid immune evasion within the host (Zhang and Norris, 1998; Barbour et al., 2006; Coutte et al., 2009; Graves et al., 2013; Norris, 2015) 	<ul style="list-style-type: none"> • <i>visE</i> system generates antigenic variability to evade host immunity (Zhang and Norris, 1998; Coutte et al., 2009; Graves et al., 2013; Norris, 2015; Tufts et al., 2019) 	<ul style="list-style-type: none"> • <i>Vmp</i> system generates antigenic variability to evade host immunity (Barbour et al., 2006)

consequences arising from these past evolutionary changes.

Our current understanding of the evolutionary genetics of *Borrelia* species can be informed by investigations into the ecological interactions encountered by *Borrelia* species during their life cycle. These interactions are the product of past evolutionary processes that have shaped the observable genotypic and phenotypic diversity. Many investigations have focused separately on either the evolutionary history or the ecology of several *Borrelia* species, both of which have been invaluable to our understanding of the biology and history of *Borrelia*. Studies focusing on the evolutionary history of *Borrelia* have detailed genetic and phenotypic variation across space and among host species, genetic and genomic diversity and divergence as well as the processes occurring over millennia that have resulted in the variation within and between species observed today (De Michelis et al., 2000; Dykhuizen et al., 2008; Graves et al., 2013; Coipan et al., 2018). These evolutionary patterns and processes have resulted in molecular factors associated with host and vector specialization or mechanisms of escaping immune responses, differences in the rates and processes leading to diversification, and the identification of virulence factors that enhance survival or transmission (Pal et al., 2001; Fingerle et al., 2007; Neelakanta et al., 2007; de Silva et al., 2009; Kenedy et al., 2012; Ogden et al., 2015; Tufts et al., 2019); many of these factors are directly linked to host and vector specialization. Ecological studies have, on the other hand, characterized processes and interactions affecting the distribution and abundance of *Borrelia* species (Paul et al., 2016; Kilpatrick et al., 2017; Vuong et al., 2017). Connecting these ecological processes and interactions with the resulting evolutionary adaptations can reveal how these evolutionary changes have shaped and will continue to shape current and future ecological interactions and species distributions.

The common ancestor of all *Borrelia* species appears to be an obligate parasite that interacted exclusively with or within vector and host environments. Thus, vectors and hosts constitute the selective environment to which *Borrelia* must adapt to maximize survival and transmission efficacy (Haven et al., 2011). The disparate selective pressures experienced by species of *Borrelia* or populations within a species that interact with different vector or

host species have resulted in divergent molecular adaptations that can lead to increased specialization on a subset of vectors or hosts. In many cases, the increased specialization feeds back, increasing selective pressures on other molecules to increase the efficacy of their interactions with molecules in some hosts or vectors at the cost of decreasing interaction efficacy in other host or vector species (Wang et al., 2001; Tufts et al., 2019). Associations with a limited subset of host or vector species further drive micro-evolutionary changes in *Borrelia* populations and species by shaping patterns of gene flow and geographic distributions (McCoy et al., 2013; Khatchikian et al., 2015; Seifert et al., 2015) as well as altering the effects of genetic drift and mutation (Brisson et al., 2012).

Investigating the complex web of ecological interactions among *Borrelia*, their vectors, and their hosts, facilitates detection of both the selective pressures that have given rise to the present-day genetic variation and the downstream evolutionary changes that are a product of that variation. Although the evolutionary and ecological history of Lyme borreliosis have been the subject of extensive investigation (Kurtenbach et al., 2006; Margos et al., 2011; Brisson et al., 2012; Ogden et al., 2015; Kilpatrick et al., 2017), significantly less research has focused on the relapsing fever group (Cutler, 2010; Talagrand-Reboul et al., 2018). Here, we synthesize and compare recent findings on the evolutionary genetics from both groups of *Borrelia*, with a focus on identifying ecological processes that have driven adaptations and the current distribution of genetic variants within and between *Borrelia* species and the evolutionary consequences of that variation. Understanding the feedbacks between the evolution of these species and their ecological interactions is critical for predicting and controlling future disease epidemics.

Interactions between *Borrelia* and their vectors

Evolutionary impact of vector specialization

All *Borrelia* species depend on at least one vector species for the completion of their life cycle. Interactions between *Borrelia* and its vectors select for molecules that enable successful uptake from infected hosts, persistence within the vector, migration through vector tissues, and transmission and persistence in subsequent vertebrate hosts (Pal et al., 2001; Fingerle et al., 2007; Neelakanta et al., 2007; de Silva et al., 2009; Kenedy et al., 2012). Additionally, many RF *Borrelia* species have

molecular machinery that enables transovarial transmission (Barbour and Hayes, 1986; Cutler, 2010; Rollend et al., 2013), although these molecules are not well characterized. For example, all studied LB species express the Outer Surface Protein A (OspA) which binds to the *Ixodid* tick midgut receptor, TROSPA, and enables colonization and persistence within the tick (Pal et al., 2004; Battisti et al., 2008). *Borrelia* specializing on different tick species experience different selective pressures at the molecular level owing to variation in tick proteins (like TROSPA) that *Borrelia* must exploit to complete its enzootic cycle (Konnai et al., 2012). That is, different tick environments impose different selective regimes on *Borrelia* traits, resulting in refinement by natural selection to maximize efficacy of each *Borrelia* species in a single vector species (Couper et al., 2020). The molecular variants resulting from this natural selection create variation in vector competence and vector specialization which further influences downstream interactions between *Borrelia* and vertebrate hosts. Molecules mediating persistence and specialization of RF *Borrelia* species within vectors have not been investigated to a similar depth. Nevertheless, most LB and RF *Borrelia* species are transmitted by a single species or related group of ticks such that the genes that mediate these interactions are expected to vary little within *Borrelia* species (Wilske et al., 1993; Eisen, 2020).

Vector associations are important drivers of the geographic range and migration rates of *Borrelia* species (Margos et al., 2011, 2019a). Variation in home ranges and rates of gene flow among *Borrelia* species can be attributed both to the feeding patterns of their vectors and to the range and mobility of host species parasitized by their vectors (Vial et al., 2006; Vial, 2009; Margos et al., 2011; Vollmer et al., 2011a; Stanek et al., 2012; Trape et al., 2013; Norte et al., 2020a). As an adaptation to their endophilic lifestyle, *Argasid* ticks like *Ornithodoros sonrai* typically take rapid bloodmeals to avoid predators and exposure to harsh environmental conditions outside of their sheltered microhabitats (Vial, 2009). Shorter feeding times in combination with feeding preferentially on territorial, burrowing vertebrates significantly constrains opportunities for migration and gene flow of both *O. sonrai* and *B. crocidurae*, the RF species that it vectors (Vial et al., 2006). Geographically isolated subpopulations of many RF *Borrelia* species vectored by ticks that parasitize less-mobile host species are genetically divergent due to both neutral evolution and to natural selection favoring

specialization to local environmental conditions (Vial et al., 2006; Hoen et al., 2009; Humphrey et al., 2010). In contrast, *Ixodid* ticks remain attached to their hosts for several days and some, like *I. dentatus* or *I. uriae*, preferentially feed on mobile host species like birds (Sonenshine, 1979; Eveleigh and Threlfall, 1974.). LB species associated with vectors that feed on highly-mobile host species exhibit limited population genetic structure as a result of frequent migration among subpopulations (Margos et al., 2011; Vollmer et al., 2011b; Gómez-Díaz et al., 2011). Although these *Borrelia* species may experience a wider range of ecological interactions with more diverse host communities, they rarely show divergent features across their geographic range (Norte et al., 2020b). Thus, despite experiencing more diverse ecological interactions, *Borrelia* species with broad geographic ranges tend to harbor less genetic variation than *Borrelia* species exposed to fewer ecological interactions due to the homogenizing effect of gene flow among subpopulations.

Borrelia that colonize a novel vector species are exposed to different selective pressures derived from distinct ecological interactions in dissimilar geographic ranges. This can result in genetic divergence between populations of the same *Borrelia* species vectored by different arthropod species. For example, as populations of the RF species *B. duttonii* adapted to the body louse, *Pediculus humanus*, they diverged from the ancestral populations vectored by *O. moubata* (Lescot et al., 2008). Following this vector switch, the louse-borne populations experienced a massive geographic expansion due to their close association with human populations. Further, genetic data revealed sufficient evolutionary divergence and genome degradation to classify these populations as a distinct species, *B. recurrentis* (Lescot et al., 2008). Similarly, a vector switch by *B. bavariensis* from *I. persulcatus* to *I. ricinus* provided an opportunity for the *Borrelia* species to expand into Europe (Gatzmann et al., 2015; Becker et al., 2016). Vector switching not only allows *Borrelia* species to invade novel ecological niches, the resulting genomic diversification can result in speciation.

Despite the generally tight associations between *Borrelia* and their vectors, the direction and rate of gene flow of *Borrelia* species typically correlates only weakly with those of their vectors at fine temporal and geographic scales (Walter et al., 2017). This is at least partially due to the fact that vertebrate hosts frequently transport *Borrelia* spirochetes in the

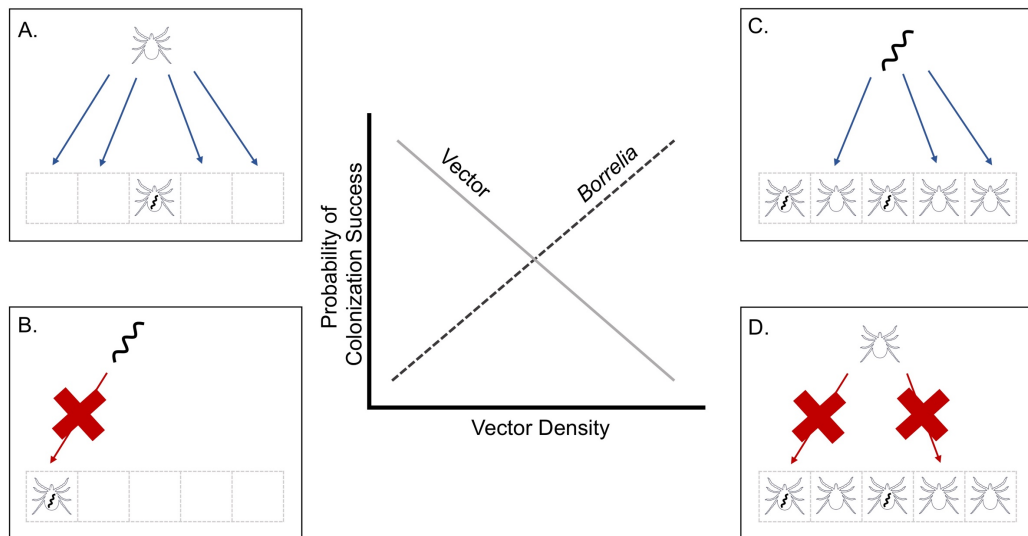


Figure 1. Differences in the rates of gene flow between *Borrelia* and their vectors may be impacted by differences in colonization efficiency. The rate at which *Borrelia* species and their vectors colonize new sites could be dependent, in part, on the size of the resident vector population. **A.** Vectors dispersing into areas with small resident vector populations may colonize with high efficiency due to limited competition with resident vectors if intraspecies competition imparts colonization efficiency, **B.** while migrant *Borrelia* spirochetes are likely to colonize with low efficiency in these areas due to the limited opportunities for transmission. **C.** By contrast, there are many transmission opportunities for *Borrelia* at locations with large vector populations that could result in higher colonization efficiency. **D.** whereas large resident vector populations could limit the probability that dispersing ticks will colonize due to competition with resident vectors. These differences in colonization efficiencies between *Borrelia* and their vectors, mediated by the size of resident vector populations, may have contributed to the observed differences in the rate and direction of gene flow between *Borrelia* species and their primary vectors. Additional research on the impact of intraspecies competition on population dynamics and colonization efficiency is necessary to determine the impacts of competition on the evolutionary history of both *Borrelia* and vector species.

absence of their vectors and vice versa. Namely, infected hosts can transport *Borrelia* even when they are not being parasitized by vectors, and uninfected hosts can transport uninfected vectors in the absence of *Borrelia*. For example, while soft ticks like *O. turicata* typically take short blood meals and have limited inter-burrow movement, *B. turicatae*-infected hosts may easily migrate between burrows (Adeyeye and Butler, 1989), and leave their bacteria in a new location. Likewise, population genetic structure among circulating *B. garinii* strains has been shown to correlate strongly with the local genetic structure of their tick vector *I. uriae* that diverge into distinct host races exploiting different sympatric seabird species at the scale of a colony (McCoy et al. 2013). However, no structure in *B. garinii* strains is evident at the scale of the ocean basin due to the large-scale movements and local colonization dynamics of the

bacterium with the seabird host (Gómez-Díaz et al. 2011). The rates and direction of gene flow may differ between *Borrelia* species and vectors even if they disperse together because the bacterium and vector colonize locations at different efficiencies (Figure 1). That is, *Borrelia* may colonize new sites with high efficiency when the local density of vectors is high, while competition with resident vectors may limit the efficiency by which immigrating vectors can establish at these new sites. Additional work is needed to reveal how migration within vertebrates and differential colonization efficiencies contribute to the observed differences in the rate and direction of gene flow between *Borrelia* and their vectors (Walter et al., 2017).

Evolutionary genetic consequences arising from interactions with the vector environment and co-infecting pathogens

The evolutionary genetics of *Borrelia* populations are shaped by many of the environmental features experienced by their vectors. Survival of *Borrelia* within non-homeothermic ticks poses a significant challenge owing to exposure to daily and seasonal temperature fluctuations in addition to the physiological changes caused by environmental fluctuations (Kung et al., 2013). Experimental evidence from other bacterial species has demonstrated that fluctuating conditions select for microbes that are tolerant of a wider range of environments due to the strong selective pressure for tolerance resulting from extreme environmental variation (Chevin and Hoffmann, 2017; Saarinen et al., 2018). The extraordinary environmental fluctuations with which *Borrelia* interacts have even led some to hypothesize that *Borrelia* may have adapted to infect vertebrate hosts as a means of escaping environmentally hostile conditions that negatively impact vector populations (Cutler, 2010). Interestingly, RF *Borrelia* species vectored by nidicolous ticks have been shown to survive and remain infectious within unfed ticks for several years, possibly owing to the fact that their vectors predominantly reside within burrows, caves, and human and livestock habitats, buffered against environmental fluctuations (Francis, 1938). By contrast, all LB species within *Ixodid* ticks remain exposed to environmental extremes of both the summers and winters in temperate climates and experimental studies have shown that LB survival in ticks may strongly decline at high temperatures (Shih et al., 1995). Understanding the mechanisms by which *Borrelia* withstand wide-ranging environmental fluctuations may provide insights into how recent and future changes in climate will ultimately impact their geographic distribution and evolutionary trajectories.

Borrelia and other microbes within their vectors may facilitate or compromise one another's ability to colonize, grow, and be transmitted to hosts (Diuk-Wasser et al., 2016; Moutailler et al., 2016; Ross et al., 2018). For example, several *Borrelia* species have been found to co-occur with a variety of other human pathogens, including the causal agent of human babesiosis, *Babesia microti* (Diuk-Wasser et al., 2016). *B. burgdorferi* sensu stricto (Bbss) is thought to facilitate *B. microti* within tick vectors as the number of ticks coinfecting with these two pathogens is higher than would be expected by

chance (Hersh et al., 2014; Diuk-Wasser et al., 2016). Coincidentally, the range and prevalence of *B. microti* has increased significantly in the northeastern United States where Bbss was already highly prevalent, suggesting important epidemiological consequences of this interaction (Dunn et al., 2014; Diuk-Wasser et al., 2016). However, for now, it remains unknown as to whether interactions between these pathogens within the tick vector are neutral or positive nor whether *B. microti* may reciprocally facilitate Bbss transmission. Further work on the nature of interactions between co-infecting infectious agents in the tick vector is now needed to better understand the role of the vector in determining the epidemiological dynamics of co-infecting pathogens.

Borrelia may also encounter other *Borrelia* species or conspecific strains within their vectors (Moutailler et al., 2016). Ticks infected by multiple strains of LB species have the same overall spirochete load as single-strain infections, suggesting there is competition among coinfecting strains which results in lower infection intensity on each strain within a tick (Walter et al., 2016; Durand et al., 2017; Genne et al., 2018; Genné et al., 2019). Since *Borrelia* density within ticks positively correlates with the probability of successful transmission to vertebrate hosts (Rego et al., 2014), competition among coinfecting strains is expected to reduce the evolutionary fitness of each strain. While inter-strain competition is expected to select for traits like increased growth rates or the production of spiteful molecules to suppress the growth of other strains (Cattadori et al., 2008; Telfer et al., 2010; Alizon et al., 2013; Susi et al., 2015), there is no evidence from genomic surveys nor from controlled experiments that such traits have evolved. More work is needed to identify the evolutionary consequences of interactions between co-infecting strains, both within vectors and within vertebrate hosts.

The limited density and diversity of microbial communities that naturally reside within *Ixodid* ticks seems likely to exert only minimal selective pressure on *Borrelia* species (Ross et al., 2018). Contrary to initial reports of a highly diverse *Ixodid* microbiome (van Treuren et al., 2015; Zolnik et al., 2016; Abraham et al., 2017), recent studies controlling for bacterial biomass demonstrated that the diversity of tick microbiomes is actually quite low (Ross et al., 2018; Couper et al., 2019). Recent investigations of both LB and RF *Borrelia* genomes revealed far fewer genes known to be involved in interbacterial

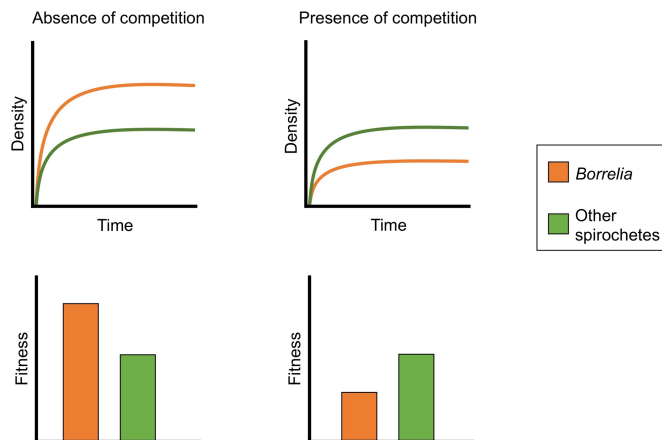


Figure 2. The presence and absence of genes involved in interbacterial interactions may have current and future fitness consequences. Many spirochetes have maintained genetic pathways for the production of molecules involved in interbacterial interaction. Most of these pathways have been lost in *Borrelia* species, likely because *Borrelia* rarely encounter rich microbiota within hosts or vectors that can reduce the fitness of *Borrelia* through competitive interactions. In the absence of competitive interbacterial interactions, the metabolic costs associated with the production of interbacterial-interaction molecules may result in negative fitness consequences for producers. The evolved loss of these interbacterial interaction pathways in *Borrelia* resulted in an increase in fitness in the absence of competition with other microbes in low diversity environments (A). In the presence of competition with other microbes (B), producers should be relatively more fit than non-producers as interbacterial interaction products provide a competitive advantage that offsets the metabolic costs of producing spiteful molecules; non-producers like *Borrelia* suffer fitness consequences as a result of their limited competitive abilities in higher diversity environments. The negative fitness consequences experienced by non-producers, like *Borrelia*, in higher microbial diversity environments may limit their ability to survive in novel environments where they are likely to encounter novel microbial species.

interaction pathways than have been found in many other surveyed bacterial species (Ross et al., 2018). Genes mediating interactions with other microbes likely have been lost in *Borrelia* owing to their limited encounters with other microbes and consequently weak natural selection for their maintenance (Zhang et al., 2012; Ross et al., 2018). This limited number of genes utilized for interbacterial interactions is likely to have negative fitness consequences for *Borrelia* species that engage in novel ecological interactions with microbes within new vectors or host species as geographic ranges change due to changes in climate or human land use (Figure 2).

Interactions between *Borrelia* and vertebrate hosts

Genetic basis and evolutionary consequences of host specialization

Interactions between *Borrelia* and their vertebrate hosts, similar to *Borrelia*-vector interactions, underlie selective regimes that favor a set of finely-tuned molecular mechanisms. These include molecules that enable vector-to-host transmission, colonization

and spread to distal vertebrate tissues, evasion of the host immune system, and transmission back to the feeding vector (Kenedy et al., 2012; Ogden et al., 2015; Tufts et al., 2019). *Borrelia* species that interact with different sets of host species experience different selective pressures at the molecular level owing to variation in vertebrate immune components and other host molecules with which *Borrelia* must interact to complete its enzootic cycle (Roberts et al., 1998; Hammerschmidt et al., 2014a; Hart et al., 2018; Tufts et al., 2019). The sequence variation and molecular determinants of host associations in the LB group have been studied at much greater depth than those in the RF group, although it is likely that similar processes have occurred in both groups. Among LB species, even minor genetic differences between orthologous proteins can substantially impact resulting interactions with vertebrate host molecules. For example, molecular divergence at the complement regulator acquiring surface protein A (CspA) results in differential ability to bind immune components and establish infection within various vertebrate species (Hart et al., 2018; Lin et al., 2020).

The genetic sequences and related molecular processes in each *Borrelia* species ultimately feedback to shape the strength and frequency of subsequent interactions between *Borrelia* and the vertebrate host community which then impacts their geographic distribution, population genetic structure, and ensuing opportunities for interactions.

Host associations occur when vertebrate host species are infected more often than expected given their frequency in the host community. These associations are common among both LB and RF *Borrelia* species and are even emerging among *Borrelia* species previously considered to be host generalists (Hanincová et al., 2006; Kurtenbach et al., 2006; Mechai et al., 2016). For example, while Bbss infects a wide range of vertebrate species, individual strains clearly have host associations, as they do not all colonize and survive within the full range of vertebrate hosts (Anderson et al., 1990; Barthold et al., 1991; Norris et al., 1995; Wang et al., 2002; Barbour et al., 2009; Baum et al., 2012; Chan et al., 2012), and they vary in their capacity to infect and cause disease in humans (Dykhuizen et al., 2008; Wormser et al., 2008; Hanincova et al., 2013). The sequence variation among vertebrate species at molecules such as plasminogen and complement regulator proteins, molecules with which Bbss must interact to successfully colonize a host, limits the range of host species each Bbss strain can infect (Ripoche et al., 1988). As a result, sequence variation among Bbss strains at the molecules mediating these interactions, like OspC and OspE, produce species-specific differences in binding efficacy to plasminogen and complement regulator molecules (Lagal et al., 2006; Önder et al., 2012; Tufts et al., 2019). Since each Bbss strain maintains just a single OspC sequence, it can effectively bind plasminogen from only a narrow range of host species (Marconi et al., 1993; Livey et al., 1995; Casjens et al., 2018). The strong selective pressure to maximize efficacy of immune evasion imposes a major barrier to the long-term maintenance of host generalism in *Borrelia*.

The evolution of host associations is shaped by the strength and frequency of interactions between each *Borrelia* species, or individual strains within species, and each vertebrate species during its evolutionary history. As noted above, variation in host immune components creates disparate selective pressures for invading *Borrelia* species (Brisson and Dykhuizen, 2004; Bäumlér and Fang, 2013). Hosts which are

frequently infected and from which *Borrelia* achieve high host-to-tick transmission rates exert significantly stronger selective pressure than hosts that *Borrelia* rarely encounter or from which *Borrelia* are rarely transmitted to feeding ticks (Ripoche et al., 1988; Tufts et al., 2019). Among both LB and RF *Borrelia* species, the frequency of interactions with each vertebrate host is heavily influenced by the range and feeding preferences of their primary vectors (Margos et al., 2011; Talagrand-Reboul et al., 2018). As a *Borrelia* species or strain becomes increasingly well-adapted to survive and transmit from a particular host species, its ability to survive and transmit from other vertebrate species tends to diminish, constraining downstream ecological interactions (Anderson et al., 1990; Barthold et al., 1991; Norris et al., 1995; Wang et al., 2001, 2002; Barbour et al., 2009; Baum et al., 2012; Chan et al., 2012; Tufts et al., 2019).

While host associations limit the geographic range of *Borrelia* species and constrain the range of ecological interactions, adaptation to novel host species – often termed a host range expansion or a host switch – can expand the diversity and range of ecological interactions *Borrelia* experience. This has resulted in genetic divergence and even speciation among LB species. Novel host species can impose distinct selective pressures while also providing opportunities for expansion of geographic range and alternate routes and rates of gene flow. For example, evolutionary divergence between the sister species *B. garinii* and *B. bavariensis* likely resulted from the evolution of different host associations, with *B. garinii* specializing on birds and *B. bavariensis* specializing on small mammals (Margos et al., 2009; Becker et al., 2016). The divergent selective regimes imposed by the different sets of vertebrates infected by each species, along with neutral evolutionary divergence in populations isolated in different sets of host species, led to in the genetic differentiation and speciation among these specialized LB species.

Genetic differentiation among *Borrelia* adapted to different host species is a product of both a reduction in gene flow and alternate selective pressures. That is, opportunities for genetic exchange among *Borrelia* species with different host associations will be limited by a lack of proximity as members of different *Borrelia* species are rarely found infecting the same host (Dykhuizen and Baranton, 2001; Bunikis et al., 2004). In fact, rates of horizontal gene transfer among LB species are 50 times lower than the rates of horizontal gene transfer among strains within LB

species (Jacquot et al., 2014), possibly as a result of limited proximity of different *Borrelia* species associated with different host species which can result in different *Borrelia* species infecting different individual ticks (Hildebrandt et al., 2003; Brisson and Dykhuizen, 2006; Herrmann et al., 2013). *Borrelia* species also experience very limited selective pressure from vertebrate species that they encounter infrequently or those to which they are not well-adapted and thus are not competent reservoirs. The lack of selective pressure to maintain the genetic and molecular variants necessary for a *Borrelia* species to infect these non-reservoir vertebrate host species eliminates evolutionary constraints, allowing specialization on the limited set of species that serve as regular hosts (Kurtenbach et al., 2006; Becker et al., 2016; Margos et al., 2019b). This positive feedback loop, along with the limited homogenizing effect of horizontal gene transfer among *Borrelia* species, has likely contributed to the high frequency of *Borrelia* species that utilize only a limited set of vertebrate species to complete their enzootic cycles.

Host associations shape the distribution and population genetic structure of Borrelia species

Vertebrate migration patterns shape the population genetic structure and geographic distribution of LB species. LB associated with small rodents and other less mobile host species tend to have well-defined population genetic structure as a result of limited opportunities for gene flow between isolated subpopulations (Victorino et al., 2008; Vollmer et al., 2011b; Norte et al., 2020b). In contrast, LB associated with highly-mobile host species, like birds, have limited population genetic structure and few distinct subpopulations as host movement and migration facilitate genetic exchange between geographically distant regions (Norte et al., 2020b). For example, population genetic structure can be observed even at very fine spatial scales for the rodent-associated *B. afzelii*, whereas genetic structure only began to emerge at inter-continental scales for the bird-associated *B. valaisiana* and *B. garinii* (Vollmer et al., 2011b, 2013; Gómez-Díaz et al., 2011). Thus, *Borrelia* species associated with mobile host species maintain genetic cohesion across their geographic range, while *Borrelia* associated with less mobile host species become genetically fragmented as a result of natural selection and adaptation to local conditions or host communities as well as genetic drift within the small effective subpopulations (Vollmer et al., 2011b; Norte et al., 2020b).

The extent to which vertebrate hosts can drive changes in the geographic range of *Borrelia* species is, of course, dependent upon the geographic distribution of competent vector species. As infected *Ixodid* ticks feed for up to several days (Šimo et al., 2017), they have many opportunities to migrate in tandem with the vertebrate hosts to which they are attached. On the other hand, most *Argasid* ticks detach from their hosts within minutes, limiting the opportunities to migrate to new sites on their hosts (Vial, 2009). As expected, *Argasid* ticks seem to have more constrained geographic ranges than their *Ixodid* relatives (Stanek et al., 2012; Trape et al., 2013), but ecological data on this tick family is still fragmentary. Since all *Borrelia* species depend on both vectors and vertebrate hosts for the completion of their enzootic cycle, the limited range and faster feeding time of *Argasid* vectors likely limits the opportunities for vertebrates to drive geographic range expansion of RF *Borrelia* species.

Interactions with diverse host species can promote the maintenance of genetic diversity within *Borrelia* populations. For example, many strains of Bbss coexist at geographic locations where the vertebrate community is highly diverse (Brisson and Dykhuizen, 2004). This is because antigenically distinct Bbss strains differ in their ability to successfully infect and be transmitted from each vertebrate species and no strain has the highest evolutionary fitness in all of the vertebrate species (Brisson and Dykhuizen, 2004; Cobey and Lipsitch, 2012). Variation among Bbss strains in host associations drives niche separation by allowing different subtypes to maximize transmission success in different hosts, thereby reducing within-host competition between strains (Brisson and Dykhuizen, 2004; Hanincová et al., 2006; Mechai et al., 2016; Vuong et al., 2017; Brisson, 2018). The presence of a diverse host community can thus promote and maintain an array of genetically divergent subtypes over the long-term.

Evolutionary genetic consequences arising from interactions with the vertebrate immune system

Interactions between *Borrelia* and the vertebrate adaptive immune system drive natural selection for antigenic variability. The selective pressure for *Borrelia* to persist within its host for the duration necessary to be transmitted back to another feeding vector has resulted in the evolution of genetic and molecular mechanisms that enable immune evasion with sufficient variation to remain effective throughout a months- or years-long infection (Tufts et al., 2019).

As infection proceeds, genetic loci encoding the immunodominant surface proteins Vmp in RF *Borrelia* species and Vmp-like sequences (VlsE) in LB species undergo frequent recombination with silent cassettes, generating novel antigens that are unrecognized by the host immune system (Zhang and Norris, 1998; Barbour et al., 2006; Coutte et al., 2009; Graves et al., 2013; Norris, 2015). Further, the genetic sequences at the silent cassettes evolve much more rapidly than the genes at other loci. This is due in part to the conservation of highly-mutable tandem repeat structures in the otherwise highly-diverged cassettes, suggesting that the ability to continuously generate novel sequence variation at this locus provides a significant fitness advantage (Lawrenz et al., 2004; Bankhead and Chaconas, 2007; Graves et al., 2013).

The adaptive immune systems of infected vertebrates also may drive balancing selection contributing to the maintenance of genetic diversity within *Borrelia* populations. For example, immune memory can create negative frequency dependent selection on *Borrelia* populations if rare genotypes have a selective advantage over common genotypes due to prior infections in the host community. That is, if immune memory to specific genotypes protects hosts from future infections of the same genotype but does not prevent infection with alternate genotypes, rare genotypes will have a selective advantage over more common genotypes. The underlying immune mechanisms necessary for negative frequency dependent selection to operate in natural populations have considerable empirical support in both RF and LB *Borrelia* species (Preac-Mursic et al., 1992; Gilmore et al., 1996; Probert et al., 1997; Barthold, 1999; Marcsisin et al., 2016). For example, mice previously infected with *B. hermsii* or *B. burgdorferi* were shown to be immune to antigenically homologous strains, but remained susceptible to infection by antigenically distinct strains (Probert et al., 1997; Marcsisin et al., 2016). Further, a recent study demonstrated that *B. afzelii* strain-specific antibodies can also be passed from mother to offspring, suggesting that the effects of immune memory may last beyond a single generation (Gomez-Chamorro et al., 2019). This type of negative frequency dependent process may enable antigenically distinct strains to be maintained within *Borrelia* populations, although no direct empirical evidence of this process operating in natural populations has been reported despite laboratory evidence that the underlying immune mechanisms

necessary to select for rare genotypes are present in several natural host species (Preac-Mursic et al., 1992; Gilmore et al., 1996; Probert et al., 1997; Barthold, 1999; Marcsisin et al., 2016).

Concluding remarks

In this review, we summarized recent findings on the evolutionary genetics of both LB and RF *Borrelia* species. While we show that much work has been done to elucidate the biology and natural history of *Borrelia*, it is clear that there are still open questions about the causes and consequences of evolutionary change in these species, many of which are poised to be answered by leveraging existing knowledge on molecular mechanisms and ecological interactions. For example, molecular studies have played a critical role in identifying the mechanisms utilized by several *Borrelia* species to establish infection, evade immune responses, and transmit between hosts and vectors (Pal et al., 2001; Fingerle et al., 2007; Neelakanta et al., 2007; de Silva et al., 2009; Kenedy et al., 2012; Ogden et al., 2015; Tufts et al., 2019). Investigations incorporating the ecological interactions between species and their hosts and vectors have revealed the selective pressures that have given rise to the observable variation in the genetic and molecular components underlying these mechanisms (De Michelis et al., 2000; Dykhuizen et al., 2008; Graves et al., 2013; Coipan et al., 2018). Further, ecological and genetic studies have revealed how variation in these molecular mechanisms has continued to shape *Borrelia* evolution, driving changes in gene flow and geographic distribution, and altering the effects of genetic drift and mutation (Brisson et al., 2012; Khatchikian et al., 2015; Seifert et al., 2015). By integrating these studies, we can move beyond cataloging variation in molecular mechanisms and ecological relationships and understand how these factors are a consequence of historical evolutionary processes and how they will continue to drive evolutionary change.

Uncovering the evolutionary genetics of a system depends critically on a firm understanding of the molecular mechanisms and ecological interactions of the species being studied. While these aspects of *Borrelia* have been the subject of extensive investigation in many LB species, they remain to be investigated in even a single RF *Borrelia* species despite their greater diversity and medical importance worldwide (Table 1). For example, little is known about the molecular and genetic underpinnings of vector or host associations in any

RF species nor the pattern or causes of population genetic structure, both of which have been central to studies of many LB species. Investigations into the molecular mechanisms and the genetic underpinnings of those mechanisms from a range of RF *Borrelia* species will permit many questions about the evolutionary genetics of RF species to be addressed. Comparing evolutionary processes among species and among the LB and RF clades will provide a better understanding of the interactions and constraints that have led to the current assemblage of species and diversity of characteristics that are observable today. Without these data, identifying the major drivers of evolution in these species will remain a significant challenge.

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