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Evolutionary ecology of Lyme *Borrelia*

Kayleigh R. O'Keeffe[1,*], **Zachary J. Oppler**[1], **Dustin Brisson**[1]

[1]Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

Abstract

The bacterial genus, *Borrelia*, is comprised of vector-borne spirochete species that infect and are transmitted from multiple host species. Some *Borrelia* species cause highly-prevalent diseases in humans and domestic animals. Evolutionary, ecological, and molecular research on many *Borrelia* species have resulted in tremendous progress toward understanding the biology and natural history of these species. Yet, many outstanding questions, such as how *Borrelia* populations will be impacted by climate and land-use change, will require an interdisciplinary approach. The evolutionary ecology research framework incorporates theory and data from evolutionary, ecological, and molecular studies while overcoming common assumptions within each field that can hinder integration across these disciplines. Evolutionary ecology offers a framework to evaluate the ecological consequences of evolved traits and to predict how present-day ecological processes may result in further evolutionary change. Studies of microbes with complex transmission cycles, like *Borrelia*, which interact with multiple vertebrate hosts and arthropod vectors, are poised to leverage the power of the evolutionary ecology framework to identify the molecular interactions involved in ecological processes that result in evolutionary change. Using existing data, we outline how evolutionary ecology theory can delineate how interactions with other species and the physical environment create selective forces or impact migration of *Borrelia* populations and result in micro-evolutionary changes. We further discuss the ecological and molecular consequences of those micro-evolutionary changes. While many of the currently outstanding questions will necessitate new experimental designs and additional empirical data, many others can be addressed immediately by integrating existing molecular and ecological data within an evolutionary ecology framework.

Keywords

Evolutionary ecology; *Borrelia* ; transmission; ecological interactions

1. Introduction

Zoonotic pathogens – those that transmit among wildlife and infect humans (Box 1)–are some of the most common causes of emerging and re-emerging infectious diseases in the

[*]Corresponding author, kokeeffe@sas.upenn.edu.

world[1,2]. Many zoonotic bacterial and viral pathogens have complex transmission cycles during which they must interact with multiple natural host species and arthropod vectors[2–4](Box 1). The bacterial genus *Borrelia* is comprised of vector-borne pathogens which infect and are transmitted from multiple vertebrate host species and can cause several highly-prevalent diseases in humans and domestic animals[5–7]. While not fully resolved, the genus has three major evolutionary groups now proposed as separate genera[8–10]; the most well-studied of these is the Lyme borreliosis clade (referred to here as the LB group), the species of which are vectored exclusively by hard-bodied ticks in the *Ixodes* genus. Here, we review research results on the LB group within an evolutionary ecology framework to explore the molecular interactions involved in ecological processes that result in evolutionary change within *Borrelia* and the ecological consequences of that evolutionary change (Box 1).

The evolutionary history and ecology of *Borrelia* have been investigated extensively. Evolutionary studies of *Borrelia* have characterized within and between species diversity, genetic and phenotypic variation across space and among host species, and the intergenerational processes underlying the observed variation[11–14]. These studies have resulted in the identification of molecular factors associated with host and vector specialization, diversification rates and processes, mechanisms of immune escape, and the importance of virulence factors to the life cycle[4,15–32]. Similarly, ecological studies have provided critical advances in our understanding of the processes and interactions that affect the abundance of some *Borrelia* species on short time scales, identifying environmental conditions and host communities that directly influence year-to-year variations in *Borrelia* populations[33–35]. While both evolutionary and ecological studies have been instrumental to our understanding of *Borrelia*, many outstanding questions can be addressed only by transcending the assumption that ecological and evolutionary processes operate on different timescales (Table 1).

An evolutionary ecology research framework integrates ecological and evolutionary timescales, incorporating feedbacks between ecological interactions and evolutionary change[36] (Box 1). Ecological interactions, like those that *Borrelia* species experience throughout their transmission cycle, create evolutionary pressures that select for traits that enhance specific components of the life cycles of *Borrelia* species[37]. These evolutionary changes, in turn, effect subsequent ecological interactions with hosts or vectors that create additional evolutionary pressures. Investigating *Borrelia* within an evolutionary ecology framework provides a foundation to evaluate how ecological interactions result in micro-evolutionary change within *Borrelia* populations and the ecological consequences of those changes. This evolutionary ecology framework can be used address questions like why some *Borrelia* species are more host-specific than others or why rates of gene flow are different among populations. While some of these questions have been addressed, further investigations within an evolutionary ecology framework would provide new insights given an evolutionary context. Such evaluations are critical for understanding, predicting, and managing disease epidemics.

Central to the evolutionary ecology of *Borrelia* species are two questions:

1. How do intra- and interspecies interactions, or interactions with the physical environment, create selective forces or impact migration rates to cause micro-evolutionary changes?
2. What are the ecological consequences – such as changes in geographic, host or vector range, abundance, or virulence – of these micro-evolutionary changes?

Evolutionary ecology provides a particularly powerful investigatory framework for infectious disease systems[38–41]. For example, an investigation of a plant pathogen system within an evolutionary ecology framework resulted in accurate identification of novel host species to which plant pathogens are likely to adapt[42]. These predictions were made by considering relatedness among hosts, their ecological traits, and their geographic distributions. Research into microbes with complex transmission cycles, like those within the *Borrelia* genus, which interact with multiple hosts and environmental conditions, is particularly poised to leverage the power derived from an evolutionary ecology framework to identify ecological processes affecting evolutionary change.

As *Borrelia* species are not free-living microbes, all molecular and ecological interactions occur with or within a vector or a vertebrate host. The web of interactions among *Borrelia*, vectors, and hosts create selection pressures that drive *Borrelia* evolution[43,44], impact migration and geographic distributions of *Borrelia*[31,32,45–49], and shape mutation rates and genetic drift[50], although the latter two evolutionary forces are difficult to discern in natural populations. Here, we describe the impacts of ecological interactions on micro-evolutionary change (Box 1) with *Borrelia* that can be deduced from independent studies of ecological interactions and molecular interactions. We then discuss future directions that can maximize the utility of an evolutionary ecology framework to identify additional ecological or molecular mechanisms that are key to the *Borrelia* life-cycle.

2. *Borrelia*-Host Interactions

2.1 Generalism to specialism

Ecological interactions between *Borrelia* and its vertebrate hosts select for evolutionarily-tuned molecular mechanisms within *Borrelia* species to enhance their ability to colonize, disseminate to distal tissues, evade host immune responses, and transmit from the host to feeding ticks[15,25,26]. Sequence variation among *Borrelia* species in the proteins that mediate these molecular mechanisms is shaped by natural selection to enhance the efficacy of interactions with host proteins that vary among vertebrate host species[15,51,52]. For example, variation in a complement regulator acquiring surface protein (CspA) among *Borrelia* species results in variation in their ability to bind complement molecules in different vertebrate hosts and thus limits the host range of each *Borrelia* species[52–58]. Ecological interactions with different host species cause each *Borrelia* species to experience unique selective pressures that result in divergent micro-evolutionary trajectories (Table 1). This evolutionary divergence has ecological consequences for *Borrelia* as it limits the subset of vertebrate hosts in which each *Borrelia* species can successfully complete its infectious cycle. A core component of evolutionary ecology is to assess these types of eco-evolutionary feedback loops. Investigating these eco-evolutionary feedback loops could address

outstanding questions such as how the frequency and strength of selection on other *Borrelia*-host interactions are impacted by host specialization or the mechanisms by which *Borrelia* species evolve to become ever more specialized.

Host associations, sometimes referred to as host specialization[59], determine the frequency and importance of ecological interactions between each *Borrelia* species and each vertebrate species[60,61]. The frequency of ecological interactions with each host species, in turn, determines the strength of natural selection imposed by each host species on *Borrelia*[15,62]. For example, variation among host species in immune system components imposes different selective regimes on invading *Borrelia* species[60,61]. The strength of these selective regimes is determined by how frequently that *Borrelia* species must evade the immune system of each host species and the host-to-tick transmission rate given a successful infection of that host[15,62]. Further, adaptations that maximize survival and transmission from one host species often limit survival and transmission from other hosts, leading to selection favoring host specialism[15,63–70]. This eco-evolutionary feedback loop favors evolution towards greater host specialization (Box 1, Figure 1). Host associations can even drive speciation events. For example, the divergence between the sister species *B. garinii* and *B. bavariensis* resulted from differential host species use; *B. garinii* specialized on bird species while *B. bavariensis* specialized on small mammals[18,71]. Subsequent genetic differentiation among these specialized *Borrelia* species arose in response to the different selective regimes imposed by the different vertebrate hosts with which they interact most frequently. An evolutionary ecology research framework can facilitate investigations into the molecular changes within *B. bavariensis* that enabled it to use a different host species by considering both how ecological interactions can cause micro-evolutionary change and how that evolutionary change impacts ecological interactions.

Adaptations that cause *Borrelia* to complete their life cycles in only a specialized subset of host species subsequently limit the frequency of interactions with other vertebrate species, thus limiting the selective pressure they experience from these non-competent vertebrate species. Further, opportunities for genetic exchange between *Borrelia* species decrease when the species are less likely to occupy the same host. Thus, genetic mutations that fix in *Borrelia* species that specialize on different host species are rarely shared through horizontal gene transfer due to limited opportunities for genetic exchange[72–74] leading to continuing evolutionary divergence among the *Borrelia* species. By contrast, horizontal gene transfer rates among genotypes within the same *Borrelia* species are 50 times greater than rates of inter-species horizontal gene transfer, at least partially due to the increased opportunity for transfer within co-infected hosts[74]. Evolutionary ecology provides a framework to investigate how eco-evolutionary feedback loops drive the high proportion of *Borrelia* species currently displaying host associations.

Host-species associations are even apparent among genetically distinct strains, or genotypes, within some generalist *Borrelia* species[59,75,76] (Box 1). Genotypes of *B. burgdorferi sensu stricto* (*Bbss*) appear to differ in their ability to complete their infectious cycles in different vertebrate hosts in nature[64–69], although these statistical associations have not been experimentally verified and are distinct, in both mechanism and strength, from those confirmed among European *Borrelia* species. Nevertheless, *Bbss* genotypes differ in their

binding efficacy to host plasminogen and complement regulator molecules from different host species due to molecular adaptations (*i.e.* OspC and OspE) [15,77,78]. Genotypes also differ in their abilities to survive and disseminate to distal tissues in a range of vertebrate hosts[63–69], both of which are critical to the ability of *Borrelia* to transmit to ticks. Evolutionary ecology provides the research foundation to experimentally evaluate the mechanisms driving any existing host association differences among *Bbss* genotypes by focusing on eco-evolutionary feedbacks between interactions with host species and the impacts of these interactions on micro-evolution.

Variation among host species in plasminogen and complement regulator molecules may require *Bbss* genotypes to adapt to only a subset of host species, resulting in interactions with molecules from other species that prevent successful infections[62]. For example, each *Bbss* genotype maintains a single OspC variant that interacts effectively with plasminogen from only a limited number of host species[79–81]. While expressing multiple OspC variants may increase the range of host species that a *Bbss* genotype can infect, it may come at the cost of increasing immune targeting[82]. OspC is targeted by a fast and lethal immune response such that more targets could increase the probability of immune clearance before the bacterium can establish a long-lasting infection. The evolutionary ecology research framework can be used to investigate if a tradeoff between host-range breadth and immune targeting imposes a barrier to the long-term maintenance of host generalism in *Borrelia* genotypes. That being said, there is growing evidence that the arrival of *Borrelia* in North America pre-dates the last Ice Age[17,24], and such a long evolutionary history may suggest that generalism is being maintained. Future work within evolutionary ecology could elucidate the apparent discord.

2.2. Interactions with the vertebrate immune system

Interactions between *Borrelia* species and the vertebrate adaptive immune system impose strong selective pressures on *Borrelia*. These interactions result in an evolutionary arms race between host immune responses and molecular mechanisms that enable immune evasion within *Borrelia* species[15]. The *vlsE* locus in all *Borrelia* species encodes an immunodominant surface protein that undergoes extensive and rapid antigenic variation during vertebrate infection in order to evade antibodies[11,83–85]. Novel *vlsE* protein sequences that are unrecognized by circulating antibodies are generated by recombination between the *vlsE* expression site and one or more of the unexpressed, highly-variable *vls* cassettes. The conservation of highly-mutable tandem repeat structures across the otherwise highly-diverged cassettes suggests that the host immune system imposes a strong selective pressure to maintain evolvability at this locus and results in ongoing micro-evolutionary change in the *vls* antigenic variation system[11].

Acquired immunity can also create negative frequency dependent selection processes that maintain genetic variation within *Borrelia* populations (Box 1). Differences among host individuals in their history of antigen exposure can result in acquired immunity to specific *Borrelia* genotypes, thus reducing the proportion of hosts susceptible to the more common *Borrelia* genotypes. Therefore, rare *Borrelia* genotypes to which few hosts have been exposed would have a selective advantage over the more common genotypes to which many

hosts have mounted an immune response. Experimental studies have also shown that genotype-specific antibodies are maternally inherited such that selection against common genotypes may last across generations[86]. Although fluctuating frequencies of genotypes are often associated with negative frequency dependent selection, *ospC*-major groups do not appear to fluctuate cyclically in natural populations[87]. Contrary to most textbook examples, however, negative frequency dependent selection on multi-strain pathogens can produce a variety of dynamics in addition to cyclical frequency fluctuations. For example, as discussed in Durand *et al.* 2017, the host immune system could cause pathogen populations to organize into communities of serotypes that minimize cross-reactive acquired immunity whose frequencies remain stable over long periods of time[88–90]. The exact mechanisms underlying this somewhat counterintuitive population dynamic under negative frequency dependent selection can be further explored within an evolutionary ecology research framework.

2.3 Borrelia distribution and genetic structure is shaped by host associations

The geographic distribution and population genetic structure of *Borrelia* species is shaped, in part, by the migration patterns of infected vertebrate hosts. *Borrelia* species associated with highly-mobile hosts (*i.e.* birds) tend to have limited population genetic structure as hosts maintain genetic cohesion over large geographic ranges[31,32,47–49]. By contrast, species associated with less mobile hosts (*i.e.* small mammals) tend to show much stronger population genetic structure, with different selective environments in different geographic areas and few opportunities for horizontal gene transfer among isolated populations[31,47,91]. For example, population genetic structure can be detected only at very large (inter-continental) scales in bird-associated species like *B. garinii* and *B. valaisiana* while the rodent-associated species like *B. afzelii* shows extensive spatial structuring even at fine spatial scales[31,32,92]. Highly disconnected populations limit horizontal gene transfer and reduces effective population sizes, which in turn, amplifies the impact of genetic drift leading to further differentiation among subpopulations.

Host species diversity can select for the maintenance of genetic diversity within *Borrelia* populations. Multiple genotypes of *Bbss* coexist within geographic locations when the environment is heterogeneous (*i.e.* a diverse vertebrate community) and none of the genotypes have the highest fitness in all host species[60,93]. Molecular variation among genotypes within *Borrelia* species determines the transmission success of antigenically distinct genotypes in each host species resulting in niche separation[33,59,60,75,94] (Box 1). The presence of multiple host species at individual locations permits an array of genetically distinct genotypes to be simultaneously maintained. As vertebrate communities have been and continue to be impacted by land use change[95], evolutionary ecology will provide a framework to predict how changes in vertebrate host communities will impact the diversity of *Borrelia* populations.

Borrelia-Vector Interactions

3.1 Vector specialization, vector switches, and *Borrelia* distribution

All *Borrelia* species require at least one tick species to complete their life cycle. Similar to the molecular interactions between *Borrelia* and its vertebrate hosts, interactions between

Borrelia and its vectors select for molecules that enable successful uptake by feeding ticks, persistence within the tick midgut throughout the tick molt and subsequent questing, and migration to the salivary glands and transmission to a subsequent host[25,27–30]. For example, Outer Surface Protein A (OspA) in *Borrelia* must bind to the tick midgut receptor, TROSPA, in order to colonize and persist within the tick[96,97]. Genes involved in interactions with ticks generally have little variation within *Borrelia* species[98], but there is substantial variation between *Borrelia* species[99]. The among-species variation observed at these genes is likely caused by differences in binding efficiency to tick proteins (*i.e.* TROSPA receptors) that likely differ among tick species[99]. Past natural selection has resulted in molecular specialization to maximize efficacy in one or a few tick species resulting in the observed differences in vector competence and vector specialization among *Borrelia* species[100]. Future evolutionary ecology research can determine how the frequency and strength of selection on other molecular interactions is impacted by vector specialization and evaluate the ecological consequences caused by the resulting micro-evolution.

Specialization to one or a few vector species impacts the geographic range and migration rates of *Borrelia* species[101,102], primarily through the mobility and range of the vertebrate species commonly parasitized by the vector. Some vectors, like *I. dentatus*, prefer mobile hosts like birds[103], while others, like *I. spinipalpis*, feed primarily on rodents with smaller geographic ranges[104,105]. As expected, *Borrelia* associated with vectors that feed on more mobile hosts have less population genetic structure due to the high rates of migration among locations[31,47,101]. In contrast, *Borrelia* species in vectors parasitizing less-mobile hosts tend to have greater genetic divergence among geographically separated populations due to both neutral evolution and natural selection favoring specialization, despite experiencing limited ecological interactions within their restricted location[24,106]. Differences in *Borrelia* population structure can stem from both direct associations with vertebrates, as discussed in the *Borrelia*-host section, or indirectly through associations with vectors that differently associate with different vertebrate species. Evolutionary ecology investigations can differentiate among potential host- or vector-associated mechanisms underlying these patterns.

Adapting to novel vector species exposes *Borrelia* populations to previously unencountered ecological interactions with both the novel vector and with the vertebrate community within the host range of the novel vector. For example, populations of *Bbss* that adapted to a new vector species, *I. pacificus*, diverged phenotypically from the ancestral populations carried by *I. scapularis* such that *Bbss* acquisition efficiency is higher in sympatric pairings of ticks and *Bbss* populations than allopatric pairings[100]. *I. pacificus*-associated *Bbss* populations also expanded geographically into the range of its new vector. While the evolutionary divergence is currently insufficient to observe geographically-based clusters when neutrally evolving loci are used to build phylogenies [107], the geographic differences in selection pressures at one or several genes have resulted in observable phenotypic differences. Adaptation to a novel vector, *I. ricinus*, also split two populations of *B. bavariensis*; one population was likely able to invade Europe by adapting to *I.ricinus* [18,108] (Figure 2). Through an evolutionary ecology lens, future research could consider the genetic mechanisms underlying this divergence and how the evolved changes influenced ecological interactions.

Although *Borrelia* species can disperse within vectors and vertebrate hosts, the direction and rate of *Borrelia* migration (gene flow) and that of their primary vector are often only weakly correlated at fine geographic and temporal scales[17]. This discord may be explained by *Borrelia* dispersing predominantly within infected vertebrates. For example, migrating birds infected with *Borrelia* may seed local tick populations in nonendemic areas[22]. *Borrelia* that do disperse within ticks may still have different rates and direction of gene flow from the tick vector if the bacteria and vectors colonize areas at different rates. That is, while the colonization efficiency of *Borrelia* is expected to be positively correlated with the local density of vectors needed to transmit the bacterium, the colonization efficiency of ticks may be inversely proportional to local vector densities due to competition with resident ticks. While the mechanisms underlying competitive interactions between ticks within or between species – as well as the impact this competition may have on colonization efficiency – require further research, recent evidence suggests that ticks do compete for rodent hosts[109]. By considering how ecological interactions impact migration rates (micro-evolution), an evolutionary ecology framework could identify the cause of the observed differences in gene flow between *Borrelia* and tick.

3.2 Interactions within ticks

Borrelia interact with other microbes within tick vectors, including other *Borrelia* species or strains, other pathogens, and non-pathogenic components of the tick microbiome[110–112]. As *Borrelia* density is positively correlated with the probability of tick-to-host transmission[113,114], the competition between *Borrelia* strains within individual ticks that reduces the density of each strain[114–117] decreases the evolutionary fitness of *Borrelia* strains. Evolutionary theory predicts that inter-strain competition that negatively impacts evolutionary fitness should select for traits that increase growth rates within ticks or for molecules that suppress competing strains[118–121]. Although *Borrelia* is often found in multi-strain infections in ticks that reduce evolutionary fitness by reducing transmission probabilities[114], there is no evidence that traits enhancing competitive ability have evolved. This may indicate that there are other important ecological interactions that are imposing selective pressures on *Borrelia* populations. Future evolutionary ecology research can experimentally or statistically investigate the selective pressures created by competitive interactions between coinfecting *Borrelia* strains, as well as other ecological interactions, to determine their relative evolutionary impact.

Borrelia can also interact with other human pathogens vectored by ticks such as *Babesia microti*, the protozoan pathogen that causes human babesiosis[111]. The number of ticks coinfected with *B. microti* and *Bbss* is higher than expected by random chance alone[111,122] suggesting that *Bbss* may be facilitating infection with *Babesia.* This facilitation may be responsible for the increased prevalence and range expansion of *Babesia* in the northeastern United States[111,123]. While facilitation of *Babesia* may occur as a byproduct of natural selection *Bbss* otherwise experiences, future evolutionary ecology research can determine the traits of *Bbss* that are key to this ecological interaction and their impacts on the evolutionary trajectories of both species.

Experimentally detecting and quantifying biological interactions between pathogens is a long-standing challenge[124–126]. Longitudinal sampling, the current gold standard for inferring ecological interactions[127,128], requires resource-intensive efforts over many years. Collecting cross-sectional data is less burdensome and can be used to identify deviations from the expected probability of coinfection given the prevalence of each pathogen[129–131]. A departure from the random expectation may indicate interactions between pathogens but may also result from environmental heterogeneity and spatial or ecological clustering[132]. Further, the assumption that the prevalence of noninteracting pathogens should be statistically independent has been challenged[124]. It is critical that ecological interactions are correctly inferred to consider them as selective forces within an evolutionary ecology framework. Appropriate analytical methods, such as those developed by Alizon *et al* 2019, can accurately infer species interactions from cross-sectional data while accounting for environmental heterogeneity but, to date, are rarely used[132].

Borrelia species encounter limited diversity and densities of bacteria within *Ixodid* ticks overall such that selective pressures from interactions with the tick microbiome as a whole should be minimal. Although early studies found diverse microbiomes in multiple *Ixodes* species and life stages[133–135], more recent studies that controlled for bacterial biomass found that the internal microbiome diversity is actually quite low[110,136]. Consistent with limited microbiome diversity and density, *Borrelia* has lost genes that encode known interbacterial interaction pathways[110] that have not been under selective pressure by resident microbes[137]. As *Borrelia* geographic ranges expand, so too does the diversity of interactions *Borrelia* experiences, as the composition of tick microbiomes varies geographically[134]. Further, there is growing evidence that the ranges of other tick-associated microbes like *Babesia* and *Anaplasma* are expanding into areas already inhabited by *Borrelia* species[138,139]. The evolutionary loss of genes encoding inter-microbial competition pathways may make *Borrelia* particularly susceptible to inhibition or exclusion by competing tick-associated microbes. Evolutionary ecology provides the foundation to determine if the evolutionarily reduced *Borrelia* genome constrains the ability of *Borrelia* to outcompete novel microbial competitors.

Borrelia populations are also shaped by the abiotic factors that affect the populations of their non-homeothermic vector (*e.g.* temperature, climate, landscape connectivity). Survival within unfed or intermolt ticks poses significant challenges owing to temperature extremes caused by daily and seasonal fluctuations as well as nutritional stress[140]. Environmental stress can create directional selection pressures for tolerance to environmental variation[141]. In fact, experimental evidence suggests that fluctuating environments select for bacteria that can tolerate a range of conditions[142,143]. Although no genes have been identified in *Borrelia* to facilitate tolerance of extreme cold during overwintering or extreme heat during summer, the regulation of many genes is temperature-sensitive[144–146]. For example, a large fraction of novel *Borrelia* sRNAs (43%) are temperature-dependent both in function and expression levels[147]. Future evolutionary ecology research into how *Borrelia* has evolved to withstand year-round fluctuations in temperature is critical to determine how *Borrelia* persists in ticks and to predict how *Borrelia* distributions may shift with climate change.

3. Future Directions

Evolutionary ecology provides a particularly powerful framework to address outstanding questions on *Borrelia* species. This framework builds upon a strong research foundation in the biology of *Borrelia* constructed from the exceptional progress in molecular mechanistic, ecological, and evolutionary biological studies. Molecular studies have identified factors associated with host and vector specialization, diversification rates and processes, and mechanisms of immune escape which reveal ecological interactions with hosts or vectors that impact the evolution of these microbial species. Ecological investigations have characterized how environmental conditions, host communities, and host/vector associations directly influence the frequency and type of molecular interactions each *Borrelia* species experiences. Evolutionary ecology offers a framework by which we can evaluate the ecological consequences of evolved traits in *Borrelia* and predict how present-day ecological processes may result in further molecular evolutionary change.

Investigations into some outstanding questions about the biology of *Borrelia* within an evolutionary ecology framework may necessitate collecting new empirical data while others can be addressed by leveraging existing data. For example, more empirical data will be needed to characterize the frequency, strength, and type of interactions between *Borrelia* and pathogens coinfecting ticks or hosts and to determine the selective forces generated by those interactions. These data are necessary to determine how competitive interactions between coinfecting pathogens create selective forces that cause micro-evolutionary changes within *Borrelia* species. Similarly, how *Borrelia* has evolved to withstand year-round climatic fluctuations necessitates additional empirical data to predict how interactions with the environment under climate change scenarios will impact *Borrelia* transmission and evolution. In many cases, however, existing data from different disciplines can be integrated to address how ecological interactions – especially those between *Borrelia* and hosts and those between *Borrelia* and vectors – create evolutionary pressures that select for a diverse set of traits. For example, integrating ecological and molecular data can identify if and how ecological interactions drive population genetic structure in natural *Borrelia* populations.

The massive influx of *Borrelia*, tick, and vertebrate genomic information[e.g.,17,148–150] can be used to discern past evolutionary processes across multiple loci that can be used to generate tractable evolutionary ecology hypotheses which can subsequently be experimentally validated. If species under different selective regimes consistently evolve distinct trait combinations, comparative genomics can be used to draw conclusions about the evolutionary impact of ecological interactions. Comparative genomic approaches have already been used in an evolutionary ecology framework to determine the genetic underpinnings of host specialization in some infectious microbes[151].

Understanding the evolutionary ecology of *Borrelia* has practical implications for predicting expansions in geographic, host, or vector ranges and the disease risk associated with these expansions. For example, predicting potential host species by their ecological and evolutionary similarities to known hosts can identify geographic areas in which populations are likely to establish[152]. Evolutionary ecology approaches are invaluable for predicting novel hosts of emerging diseases, especially those caused by pathogens that circulate among

multiple hosts like many *Borrelia* species. By overcoming the assumption that ecology and evolution operate on distinct timescales, evolutionary ecology provides broad insight into factors regulating population and community dynamics, processes critical to understanding disease dynamics.

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Box 1:

Glossary

Complex transmission: transmission that occurs through multiple hosts or vectors

Evolutionary ecology: A research framework that explicitly considers the evolutionary histories and the ecological interactions driving evolutionary change

Generalist pathogen: Pathogens that can successfully infect and transmit from a wide range of host species

Micro-evolution: the change in allele frequencies within populations or species caused by natural selection, gene flow, mutation or drift, including changes caused by horizontal gene or plasmid transfer within and between species[81].

Negative frequency-dependent selection: When the strength and direction of natural selection is a function of the relative abundance a trait variant in a population. The fitness of a trait variant increases as the relative abundance, or frequency, of the variant decreases.

Niche: set of environmental conditions in which the members of a species can survive.

Multiple niche polymorphism: Diversity within a population that is maintained because the strength and direction of natural selection on each trait variant is a function of its ability to exploit different environmental features in a heterogeneous habitat[153,154]. This type of multi-niche selection can maintain multiple trait variants in a population if each variant has a selective advantage in some available habitats while other variants are superior in other habitats.

Specialist pathogen: Pathogens that can successfully infect and transmit from only one or a small subset of possible species

Zoonotic pathogens: pathogens naturally transmitted between animals and humans

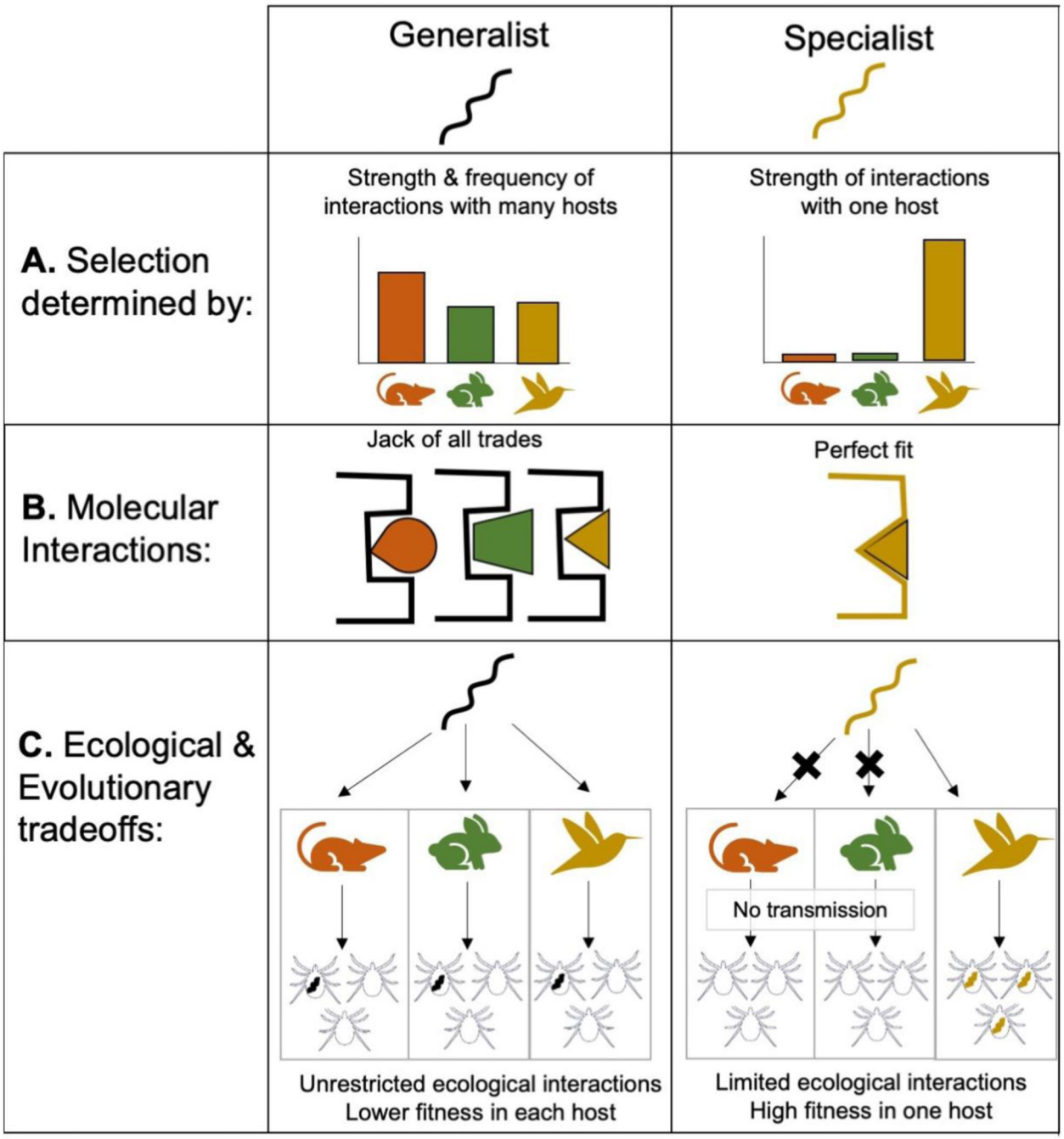

Figure 1: Why are some *Borrelia* species more host-specific than others?

Along the generalist-specialist continuum, the more generalist *Borrelia* species infect many vertebrate host species while the more specialist species infect only one or a few host species. Considering the ecological interactions each *Borrelia* species experiences elucidates the selective pressures that led to the differences in host species specialization. **A.** The strength of natural selection is derived, in part, from the frequency of ecological interactions that a *Borrelia* species experiences with each possible host species. That is, selection on generalist species is impacted by the frequency of interactions with the wider vertebrate host

community whereas selection on specialist species populations is determined primarily by interactions with the one species it can infect and from which it is regularly transmitted. **B.** The molecular mechanisms underlying successful infection vary between generalist and specialist species due to differing selective pressures. For example, the surface proteins of generalist species interact successfully but not ideally with the corresponding receptors of multiple host species, **C.** resulting in sub-optimal infection or transmission success from many species; **B.** the surface proteins of specialist *Borrelia* species interact more effectively with the corresponding receptor of its one competent host **C.** resulting in optimal infection and transmission success from only one or a small number of host species. Thus, generalist species have less restricted ecological interactions with the vertebrate community but lower fitness in each host while specialist species are limited in their interactions to the vertebrate host species it can successfully infect and be transmitted from, but experience high fitness in that host.

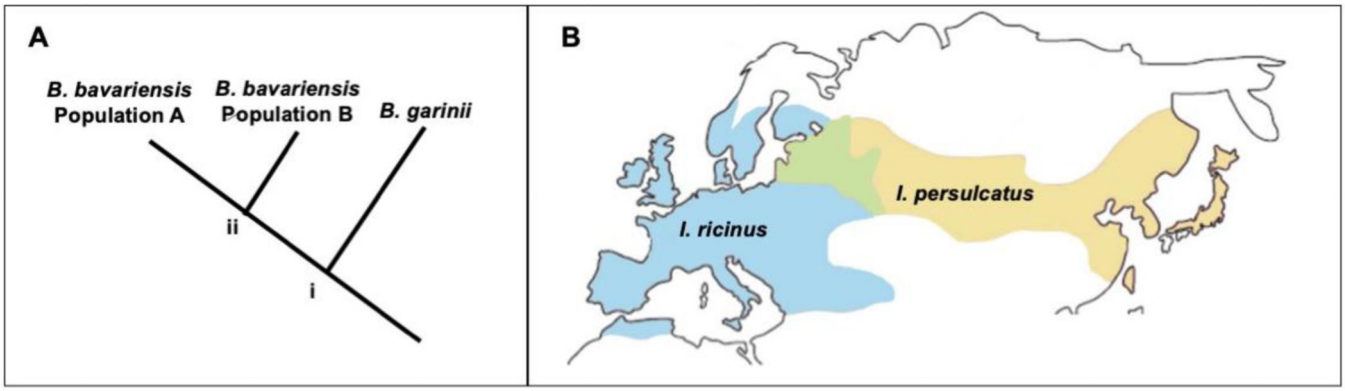

Figure 2: Diversification of *Borrelia garinii* and *Borrelia bavariensis* in Eurasia demonstrates how evolutionary changes impact the ecological interactions.

A. The sister species *B. garinii* and *B. bavariensis* diverged at node ***i*** as a result of specialization on different sets of host species with *B. garinii* specializing on bird species and *B. bavariensis* specializing on small mammals. Subsequent *B. bavariensis* divergence into two populations at node *ii* occurred due to adaptation to a novel vector, *Ixodes ricinus*, allowing *B. bavariensis* to invaded western Europe. **B.** The distributions of *I. ricinus* (blue) and *I. persulcatus* (yellow) overlap in eastern Europe (green) where the eastern *B. bavariensis* population predominates. The host and vector switches that have occurred during the evolutionary history of these *Borrelia* species has facilitated range expansion and increased the diversity of their ecological interactions. (Species ranges as described in Stanek *et al.* 2012[158]. Adapted with permission from the European Concerted Action on Lyme Borreliosis. Available at: http://www.eucalb.com/.)

Table 1:

Evolutionary Ecology Framework

	ECOLOGY	EVOLUTIONARY BIOLOGY	EVOLUTIONARY ECOLOGY
EXAMPLE QUESTIONS	• How does intraspecific diversity contribute to host-vector-pathogen interactions? • How important are multiple infections in driving disease dynamics? • How do species interactions explain the distribution and abundance of different species?	• How do pathogens co-evolve with their vectors and hosts? • By what molecular mechanisms do pathogens replicate and how does that impact pathogen evolution?	• How do ecological interactions create selective forces or impact migration rates to cause micro-evolutionary changes? • What are the ecological consequences – such as changes in geographic, host or vector range, abundance, or virulence – of these micro-evolutionary changes?
ASSUMPTIONS	• Individuals within a species or group considered identical • No evolutionary change (short timescales)	• Constant/irrelevant population densities (Hartl and Clark 1989) • Fitness in light of ecological interactions considered as constant • Most evolution is neutral	• Distribution and variance of genetic variation is constant (Holt 2005)
NECESSARY DATA & EXAMPLE APPROACHES	Necessary Data: Measures of diversity (e.g. phenotypes, genotypes, species counts, functional traits); Measures of disease progression and transmission Examples: Devevey *et al.* 2015[155], Walter *et al.* 2016[117]	Necessary Data: Sequencing (e.g. multi-locus markers, whole genome sequencing, reduced representation sequencing) Examples: Brisson *et al.* 2010[156], Becker *et al.* 2016[18]	Necessary Data: Sequencing (e.g. multi-locus markers, whole genome sequencing, reduced representation sequencing); Measures of diversity (e.g. phenotypes, genotypes, species counts, functional traits); Examples: MacDonald *et al.* 2019 *bioRxiv*[157]; Becker and Han 2020 *bioRxiv*[152]