

Reviewing molecular adaptations of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles

Jean I. TSAO^{1,2*}

¹ Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48864, USA

² Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48864, USA

(Received 11 September 2008; accepted 15 April 2009)

Abstract – Lyme borreliosis (LB) is caused by a group of pathogenic spirochetes – most often *Borrelia burgdorferi*, *B. afzelii*, and *B. garinii* – that are vectored by hard ticks in the *Ixodes ricinus-persulcatus* complex, which feed on a variety of mammals, birds, and lizards. Although LB is one of the best-studied vector-borne zoonoses, the annual incidence in North America and Europe leads other vector-borne diseases and continues to increase. What factors make the LB system so successful, and how can researchers hope to reduce disease risk – either through vaccinating humans or reducing the risk of contacting infected ticks in nature? Discoveries of molecular interactions involved in the transmission of LB spirochetes have accelerated recently, revealing complex interactions among the spirochete-tick-vertebrate triad. These interactions involve multiple, and often redundant, pathways that reflect the evolution of general and specific mechanisms by which the spirochetes survive and reproduce. Previous reviews have focused on the molecular interactions or population biology of the system. Here molecular interactions among the LB spirochete, its vector, and vertebrate hosts are reviewed in the context of natural maintenance cycles, which represent the ecological and evolutionary contexts that shape these interactions. This holistic system approach may help researchers develop additional testable hypotheses about transmission processes, interpret laboratory results, and guide development of future LB control measures and management.

Lyme borreliosis / R_0 / vector-borne / tick

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* Corresponding author: tsao@msu.edu

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1. INTRODUCTION

Lyme borreliosis (LB) is an emerging vector-borne zoonotic disease of temperate climates, with a current distribution spanning North America and Eurasia. It is caused by *Borrelia burgdorferi* and closely related spirochetes that are vectored by hard ticks in the *Ixodes ricinus-persulcatus* complex¹ [65], which feed on a variety of mammals, birds, and lizards. Although LB is one of the best-studied vector-borne zoonoses, with research spanning molecular to landscape levels, the annual incidence in North America and Europe leads other vector-borne diseases and continues to increase [11].

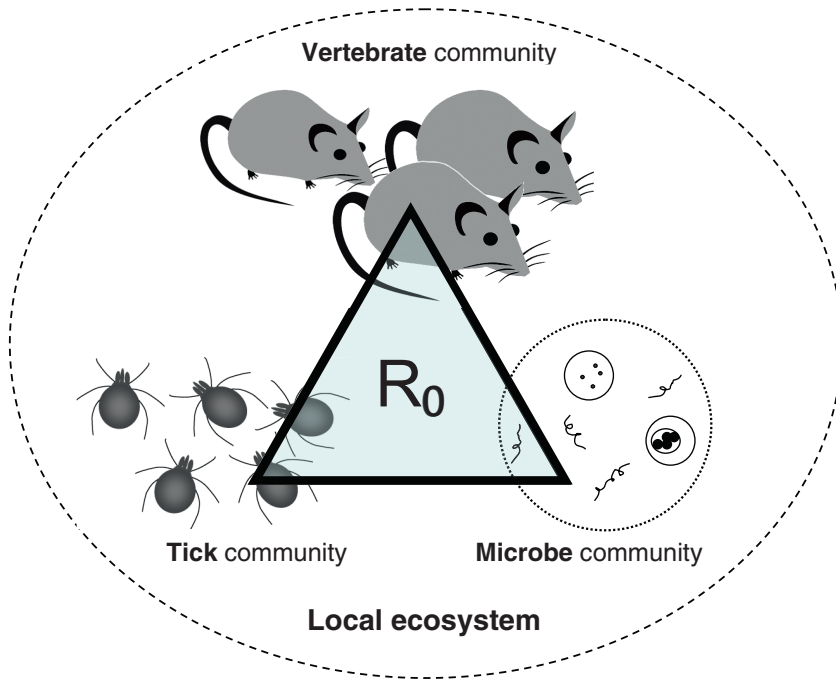
LB normally is highly treatable; however, the treatment cost and loss of productive work-days are not trivial [302]; furthermore, for some people, there are concerns of chronic disease, although the nature of chronic LB is hotly debated [62]. An effective LB vaccine is available for canines [143], but not for people [295]. Effective acaricides are available, but their application often is undesired, cost-prohibitive, or infeasible [87, 206]. Similarly, host-targeted wildlife acaricides and repellants (like those used on domestic companion animals) have been developed for deer and rodents, but are not yet widely used [36, 56, 254]. Some communities have decided to cull deer to reduce tick populations, but currently, that is not a generally acceptable or feasible solution [217]. Thus, public health agencies strive to improve

understanding of vector distributions [53], to promote awareness, and to prescribe behaviors to reduce risk of exposure, infection and disease [207]. In the USA, populations of *I. scapularis*, the vector responsible for the majority of human and canine disease, have been increasing locally and are spreading regionally, contributing to the on-going emergence of LB [11, 53]. Anticipating that demand for a human vaccine consequently will rise, researchers continue to hunt for vulnerable molecular targets of *B. burgdorferi* and the vector. There is also ongoing research to develop host-targeted and wildlife vaccines as an additional tool for integrated disease management [40, 47, 278].

Discoveries of molecular interactions involved in LB spirochete transmission have accelerated recently due to a synergy of knowledge and tools, including the publication of several Lyme spirochete and vector species genomes, advances in genetic and immunological high-throughput techniques and molecular manipulations, and experience in executing transmission experiments in well-characterized laboratory animal models. Studies reveal complex interactions among the spirochete, tick, and vertebrate triad that involve multiple, and often redundant, pathways. These reflect, in part, the evolution of general and specific mechanisms by which the spirochetes survive and reproduce.

What factors make the LB system so successful? How can researchers hope to reduce disease risk either through vaccinating humans or reducing the probability of contacting infected ticks in nature? A succession of reviews have been written about the molecular mechanisms by which *B. burgdorferi* interacts with its vertebrate and tick hosts (e.g. [33, 64,

¹ Keirans J., Oliver J. Jr., Needham G., The *Ixodes ricinus/persulcatus* complex defined, Proc. First Intl. Conference on Tick-borne Pathogens at the Vector-Host Interface, St. Paul, 1992, 302 p.



$$R_0 = \frac{N f \beta_{v-T} \beta_{T-T} \beta_{T-V} p^n F}{H (r+h)}$$

Figure 1. R_0 and the vector-pathogen-host triangle within the environment. Interactions amongst all three organisms shape the adaptations of LB spirochetes for vectorial transmission. At the heart of the triangle is R_0 , the reproductive number for LB spirochetes, which is a measure of its fitness in a given environment, where N/H is the ratio of ticks to vertebrate hosts; f is the combined probability of a tick contacting and feeding successfully on a host; β_{v-T} is the transmission coefficient for LB spirochetes from an infected vertebrate to a tick; β_{T-T} is the transstadial transmission coefficient through the molt from one life stage to the next one; β_{T-V} is the transmission coefficient for LB spirochetes from an infected tick to a vertebrate; p is the tick’s daily survival probability; n is the extrinsic incubation period; F is the vector’s fecundity; r is the host’s daily recovery rate; and h is the host’s daily mortality rate. See Appendix for further explanation.

104, 124, 151, 251, 276]). To a lesser extent, the evolutionary ecology of LB spirochetes has been reviewed in regard to its potential impacts on LB epidemiology [125]. Here, the goal is to integrate these two perspectives: to review the molecular interactions among LB spirochetes, its vectors, and vertebrate hosts in the context of natural maintenance cycles, which represent ecological and evolutionary contexts that shape the former. Furthermore, it

has been shown that the genetic diversity of LB spirochetes, which manifests as trait variation, has epidemiological and ecological consequences as well [125, 294]. Thus, a holistic system approach to examining the molecular traits of LB spirochetes may help researchers develop additional testable hypotheses about transmission processes, interpret laboratory results, and guide development of future LB control and management approaches.

Table I. Species comprising and/or potentially influencing the Lyme borreliosis biocoenosis.

Vertebrate Community	
Competent reservoir hosts	Mammals – <i>Apodemus flavicollis</i> (O), <i>Blarina brevicauda</i> (N), <i>Lepus</i> spp. (N, O), <i>Microtus pennsylvanicus</i> (N), <i>Myodes</i> [formerly <i>Clethrionomys</i>] <i>glareolus</i> (O), <i>Peromyscus leucopus</i> (N), <i>Scturus carolinensis</i> , (N,O), <i>S. griseus</i> (N), <i>S. vulgaris</i> (O), <i>Sorex</i> spp. (O, N), <i>Tamias</i> spp. (N, O)
	Birds – <i>Fratercula arctica</i> (O,N), <i>Turdus migratorius</i> (N), <i>T. merula</i> (O), <i>T. philomelos</i> (O), <i>Phasianus colchicus</i> (O)
	Lizards – <i>Eumeces inexpectatus</i> (N), <i>Lacerta agilis</i> (O), <i>L. viridis</i> (O), <i>Podarcis muralis</i> (O)
Less competent reservoir hosts	Mammals – <i>Canis latrans</i> (N), <i>Didelphis virginianus</i> (N), <i>Procyon lotor</i> (N), <i>S. carolinensis</i> (N)
	Birds – <i>Cardinalis cardinalis</i> (N), <i>Melospiza melodia</i> (N)
	Lizards – <i>Anolis carolinensis</i> (N), <i>Sceloporus undulatus</i> (N)
Incompetent hosts	Mammals – <i>Capreolus capreolus</i> (O), <i>Cervus elaphus</i> (O), <i>Dama dama</i> (N), <i>Odocoileus hemionus</i> (N), <i>Odocoileus virginianus</i> (N)
	Birds – <i>Dumetella carolinensis</i> ¹ (N), <i>Pipilo erythrophthalmus</i> (N), <i>Toxostoma rufum</i> (N)
	Lizards – <i>Elgaria multicarinata</i> (N), <i>Sceloporus occidentalis</i> (N)
Tick Community	
Bridge vectors	<i>Ixodes pacificus</i> (N), <i>I. persulcatus</i> (O), <i>I. ricinus</i> (O), <i>I. scapularis</i> (N)
Cryptic vectors, sometimes sympatric with vectors	<i>I. affinis</i> (N), <i>I. angustus</i> (N), <i>I. dentatus</i> (N), <i>I. hexagonus</i> (O), <i>I. jellisoni</i> (N), <i>I. minor</i> (N), <i>I. muris</i> (N), <i>I. spinipalpis</i> (N), <i>I. uriae</i> (O,N)
Incompetent ticks, often sympatric with vectors	<i>Amblyomma americanum</i> (N), <i>Dermacentor andersoni</i> (N), <i>D. occidentalis</i> (N), <i>D. variabilis</i> (N), <i>I. cookei</i> (N), <i>I. trianguliceps</i> (O)
Ticks of unknown competence, often sympatric with vectors	<i>I. acuminatus</i> (O), <i>I. auritulus</i> (N), <i>I. brunneus</i> (N), <i>I. marxi</i> (N), <i>I. texanus</i> (N)

Table I. Continued.

Microbe Community

Lyme borreliosis group spirochetes
(species of known pathogenicity)

Borrelia andersonii (N), *B. afzelii* (O), *B. bissetii* (N), *B. burgdorferi* (O,N),
B. californiensis (N), *B. carolinensis* (N), *B. garinii* (O)², *B. japonica* (O),
B. lusitanae (O), *B. sinica* (O), *B. spielmanii* (O), *B. tanukii* (O), *B. turdi* (O),
B. valaisiana (O), *B. yangtze* (O)

Relapsing fever spirochetes

B. miyamotoi (O,N), “*B. davisi*” (N), *B. lonestari* (N)

Other microbes

Anaplasma phagocytophilum (O,N), *Babesia* spp. (O,N),
Bartonella spp. (O,N), *Rickettsia* spp. (O,N),
flaviviruses (O,N) including tick-borne encephalitis virus (O),
louping ill virus (O), and deer tick virus (N), known and
unknown endosymbionts (O,N)

This list of species is meant to be informative and is not comprehensive. Host and tick species were selected based on common occurrence in the literature at the time of writing. Characterization of species as competent, less competent, or even incompetent is meant to illustrate the range of variation among taxa, but is not meant to be prescriptive, as competence may depend on the local community of hosts, vectors, and microbes. Microbial taxa include those known to share tick or vertebrate hosts. The geographic distributions of species are indicated generally as New (N) or Old (O) World. References for vertebrate hosts: [43, 46, 79, 81, 96, 97, 107, 121, 127, 129, 133, 141, 147, 154, 156, 157, 188, 231, 234, 266, 268, 269, 271, 282]. References for vectors: [26, 59] and references therein, [91] and references therein, [155, 201, 238]. References for microbial community: [13, 15, 20, 21, 31, 34, 39, 41, 44, 45, 68, 74, 93, 113, 131, 136, 145, 149, 152, 163, 167, 196, 199, 208–210, 232, 235, 237, 240, 241, 247, 259, 267, 272, 285].

¹ Contrary to a previous study [81], Gimsberg et al. [153] recently found xenodiagnostic larvae to be infected at a low level; the assay used, however, may not differentiate between *B. burgdorferi* and *B. miyamotoi*.

² While the epidemiological significance of *B. garinii* is greatest in Eurasia, it is associated with pelagic seabirds species that have a circumpolar distribution that even range into the southern hemisphere. In 2006 *B. garinii* was reported in *I. uriae* ticks collected off the coast of Newfoundland, making it the second LB spirochete species to be found in both North America and Eurasia [252].

This review begins with an overview of the biology and natural history of the LB system (Section 2) and then introduces the concept of R_0 , which provides the theoretical framework within which to consider spirochete-tick-host interactions with regard to spirochete reproductive success (Section 3). Getting to the heart of the article, the review showcases several spirochete-tick-host interactions at the molecular interface that appear to be key for persistence of LB spirochetes in nature (Section 4). The review then describes environmental, ecological, and evolutionary factors that may influence and shape the nature and evolution of those molecular interactions (Section 5). Finally, the review concludes with a brief discussion of questions for which a population perspective may help guide current and future research (Section 6). I aim to be illustrative, not comprehensive – furthermore, while I include examples from various systems, because of my particular experiences, the review primarily highlights the LB system in the northeastern USA.

2. BASIC BIOLOGY AND NATURAL HISTORY OF THE LB SYSTEM

Several species of spirochetes, ticks, and vertebrates participate in LB spirochete systems, which are found predominantly in northern temperate regions (Fig. 1, Tab. 1). Basic biologies of each of the three suites of organisms in relation to their maintenance of LB spirochetes in nature are reviewed. It is important to remember that their ecologies, including their interspecific interactions, are influenced by local and regional ecosystems including the abiotic factors.

2.1. The spirochete

Spirochetes are an ancient monophyletic group of bacteria – including both parasitic and free-living forms – that have a characteristic spiral shape and a unique mode of motility that enables them to swim efficiently through highly viscous media, such as connective tissue, where the movement of other bacteria is reduced or inhibited [38, 142]. The shape and motility of

LB spirochetes are provided by flagella located in the periplasmic space between the peptidoglycan layer and the outer membrane [165]. Several periplasmic flagella (PF) attach to each end of the cell, wrap around the cell cylinder and overlap at the cell center, giving the bacterium its characteristic flat-wave shape. The asymmetric rotation of the PF bundles generates backward moving waves along the cell body that propel it forward. Video footage of the dissemination of live *B. burgdorferi* spirochetes within a living mouse reveals how several types of motion are used to navigate through endothelial tissues [164].

Since *B. burgdorferi* was first described in 1984, a few years after an unusual outbreak of juvenile arthritis in Lyme, Connecticut, USA [258], more than a dozen closely related spirochetes have been identified worldwide, and more undoubtedly will be described [235]. Four species are known to cause human LB: *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *B. spielmanii* [235]. Most of the known species diversity of LB spirochetes occurs in Europe, suggesting an Old World center of origin for the group. Currently *B. burgdorferi* is the only LB spirochete that is endemic to both North America and Europe, although *B. garinii* was recently discovered in *I. uriae* ticks found along the coast of Newfoundland (North America) [252]. *B. burgdorferi* is more common in North America than in Europe where *B. afzelii* and *B. garinii* predominate [124]. While earlier studies suggested that *B. burgdorferi* was more genetically diverse in North America [67, 225], recent multilocus sequence typing analyses [150, 214] have produced contrary results and support a European origin for this spirochete, which is more consistent with the hypothesized evolutionary history of the LB group. An alternative, but not mutually exclusive, hypothesis is that recent migration of some genotypes from North America to Europe may have occurred [214].

2.1.1. LB spirochetal genomes

In order for LB spirochetes to adapt to their environments, heritable genetic variation among traits must exist. Mutation and recombination

rates, as well as the mechanisms by which genetic variants are created, are fundamental to the nature and rate of adaptive change and evolution. Thus, considering the architecture of LB spirochetal genomes can provide insight into the processes that give rise to adaptations. Additionally, comparisons among genomes can further illuminate the evolution of this group of spirochetes.

A striking feature of the published genomes of *B. burgdorferi*, *B. garinii*, and *B. afzelii* is their highly segmented organization [73, 84, 85]. At approximately 1.5 Mb, LB spirochete genomes are comprised of one linear chromosome and many linear and circular plasmids, more than any other characterized bacterium. A high degree of homology among species exists at the chromosome level, but the plasmid portion of the genomes varies greatly even among strains within a species [84, 85, 109, 274]. Plasmids encode ~40%, 29%, and 36% of the genomes of the B31 strain of *B. burgdorferi*, PBI strain of *B. garinii*, and Pko strain of *B. afzelii*, respectively [85]. Furthermore, the presence of many gene duplications, pseudogenes, and the partial or whole absence of plasmids suggest that evolution among the plasmid portion of the genome is more dynamic than on the chromosome [236, 261].

The chromosome contains many homologs of known genes, including housekeeping genes [73, 236, 261]. In contrast, many plasmid-encoded genes require experiments to infer their functions. For example, researchers have observed the phenotypic effects of the loss of partial and whole plasmids on the infectivity for vertebrates [261]. Many genes encoding putative lipoproteins are found on the chromosome, with even more on plasmids [37]. Recent studies suggest that many plasmid genes encode proteins important to spirochete reproduction, infection, transmission, and persistence in various vertebrate hosts [85, 92, 244, 298].

2.1.2. *LB spirochete persistence in I. ricinus-persulcatus ticks in nature*

LB spirochetes are maintained in nature by *I. ricinus-persulcatus* complex ticks, although other ixodid ticks participate in enzootic

maintenance (see below) [296]. *I. ricinus-persulcatus* complex ticks have three post-embryonic developmental stages – larva, nymph and adult. With the exception of the adult male, each life stage requires a blood meal from a vertebrate host. LB spirochetes primarily are maintained in nature through horizontal transmission between their tick and vertebrate hosts (Fig. 2). A naïve larva first becomes infected through an infectious blood meal. The spirochete is maintained transstadially (i.e. through the molt), and the transmission cycle is completed when infected nymphs transmit LB spirochetes back to other reservoir hosts during their blood meals. Transstadial transmission requires that spirochetes survive a number of physiological events, including blood meal digestion and molting.

It is believed that transmission from infected nymphal ticks of one cohort to larval ticks of another via reservoir hosts is largely responsible for LB spirochete maintenance in nature. This is hypothesized because adult ticks usually feed on deer or other ungulates, which are incompetent hosts, and because the ecological importance of vertical, or transovarial, transmission ranges from undetermined (*I. ricinus* systems) to negligible (*I. scapularis* and *I. pacificus*-driven systems) [198]. Because of the high fecundity of female ticks, however, the contribution of vertical transmission to LB spirochete persistence may be important in some LB systems [219].

Non-systemic, or co-feeding, transmission represents a third potential route by which infected ticks may infect naïve ticks [220]. In non-systemic transmission, spirochetes recently deposited into the feeding lesion by an infected tick may infect another closely, or sometimes subsequently, feeding tick. Non-systemic transmission has been demonstrated experimentally in the laboratory, but its relative importance in nature, especially in the presence of systemic transmission, is unknown [180, 197, 205].

2.2. The ixodid tick vector

Several tick species may vector LB spirochetes in nature. Bridge vectors are those that feed on both wildlife and humans and consequently, can present a direct zoonotic risk. In North America, the bridge vectors are

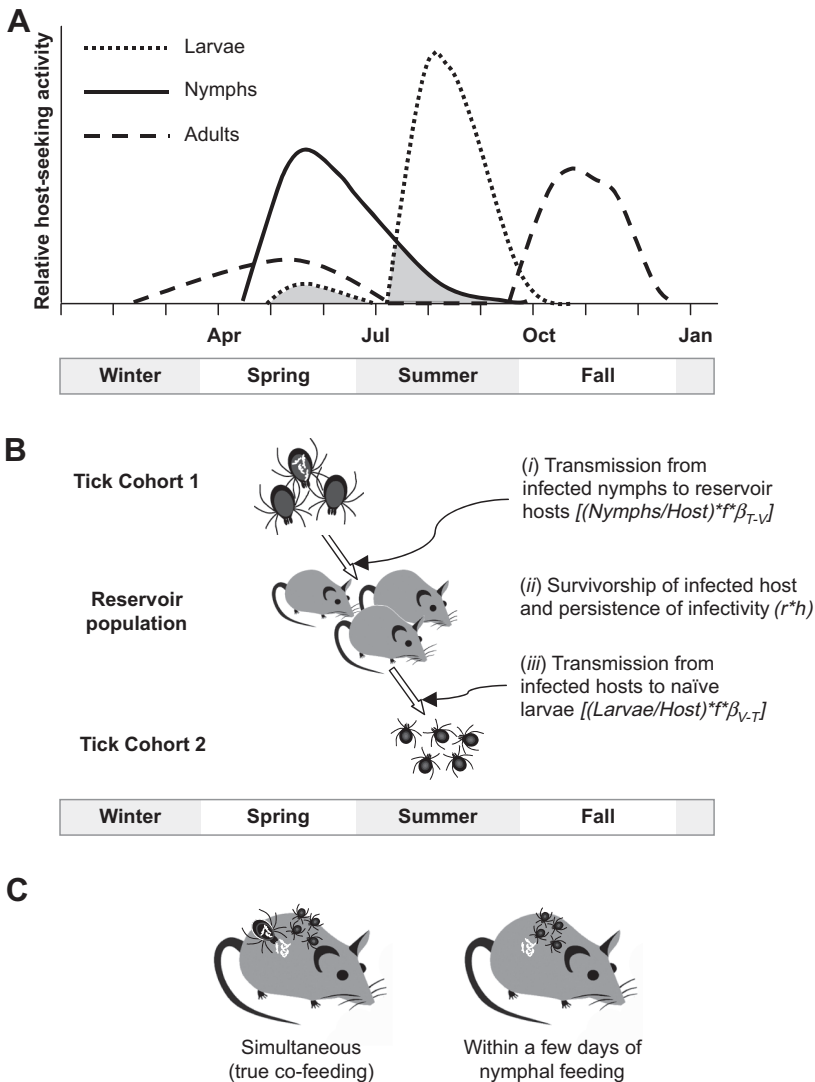


Figure 2. (A) *I. scapularis* phenology in southern New England, Northeastern USA, based on Fish (1993) [66] (Westchester County, NY, USA) and Tsao unpublished data (New Haven County, CT, USA). (B) Seasonal timing of *horizontal* pathogen transmission from nymphs (cohort 1) to larvae (cohort 2) via a shared reservoir hosts. Also noted are the relevant parameters influencing spirochete transmission as modified from Randolph and Craine [219]; see text for explanation. (C) *Non-systemic transmission* may potentially occur when naïve larvae feed either simultaneously with or very soon after an infected nymph has fed; larvae must feed near the source feeding lesion. Shaded areas of the larval and nymphal host-seeking phenology curves in (A) indicate the period over which non-systematic transmission may occur.

I. scapularis (midwestern and eastern) and *I. pacificus* (western). In Europe, the bridge vector is *I. ricinus*, whose northeastern popula-

tions overlap with that of *I. persulcatus*, a bridge vector that extends far into Asia. In contrast to bridge vectors, other ticks, both within

and outside the *I. ricinus-persulcatus* complex, can serve as enzootic, or “cryptic”, vectors; these rarely present a zoonotic risk because they rarely feed on humans (although they may feed on and infect companion animals such as dogs). The relative importance of enzootic vectors for contributing to the maintenance and diversity of LB spirochetes is undetermined.

2.2.1. Host finding and host associations

Both nest-associated (nidicolous or endophilic) and environment-associated (non-nidicolous or exophilic) species exist in the *I. ricinus-persulcatus* complex. The zoonotic vector species are exophilic and use an “ambush” strategy for contacting vertebrate hosts. They climb atop vegetation and wait for a passing vertebrate host to which to attach. At the simplest level, the likelihood of contact is a function of tick questing behavior, host abundance, and host behavior. Research on various *I. ricinus-persulcatus* complex ticks shows that they probably use several cues, including light, CO₂, thermal, and host chemicals, to increase the probability of contacting hosts [255]. Genetic factors may also play a role.

The zoonotic vectors of LB are generalists and feed on a wide range of vertebrates, including mammals, birds, and lizards [59]. The spectrum of vertebrate host species, however, often varies among different life stages. Experiments suggest that abiotic factors influence questing height, thereby influencing host species use [222]. Immatures, which are more prone to desiccation, often host-seek low in the vegetation, where they can contact hosts of all sizes. Larvae feed on mammals of all sizes, ground-feeding birds and lizards. Adults, in contrast, host-seek higher in the vegetation and therefore parasitize mainly medium-sized and larger mammals [117]. Some tick species feed only on particular vertebrate species. For example, *I. dentatus* vectors *B. burgdorferi* in North America in enzootic cycles between birds and rabbits [270]. Even more specialized, *I. uriae* vectors *B. garinii* among pelagic colonial seabirds [188]. Indeed, population genetic studies of *I. uriae* in heterospecific seabird colonies suggest the sympatric existence of “host races”, i.e.

subpopulations of *I. uriae* that feed only on particular seabird species [158, 159].

2.2.2. Feeding and development

Using toothed chelicerae, ticks cut into the host’s epithelial layers to create a feeding lesion, where they feed in a mire of fluid and blood [255]. Feeding is a gradual process during which periods of bloodsucking and salivation alternate. The completion of the blood meal requires several consecutive days, and its length varies with life stage, host species, and host immune status. For example, *I. scapularis* larvae feed for 3 to 5 days, nymphs for 4 to 5 days, and adult females for 7 days [66, 277]. Larvae and nymphs ingest approximately 10 to 20 times, and females 100 to 120 times, their unfed body weights [255]. Feeding is followed by a much longer off-host period during which engorged adult females lay a single batch of approximately a few thousand eggs, and engorged larvae and nymphs molt to the next life stage [12, 90]. The developmental processes take weeks or months to complete and are influenced mainly by temperature-dependent and temperature-independent diapause [58, 223]. Life cycles in the *I. ricinus-persulcatus* complex range from 2 to 6 years, only a fleeting proportion of which is spent on a host [5].

2.2.3. Dispersal and geographic range expansion

No life stage of ticks in the *I. ricinus-persulcatus* complex crawls more than a few meters while questing [35, 61, 89, 255]. Dispersal, both locally and more distantly, therefore depends entirely on the movement of vertebrate hosts and their behavior. For example, migratory birds and hosts with large home ranges (e.g. deer) should promote faster range expansion of ticks (and the pathogens they carry) than will hosts with smaller home ranges (e.g. mice) [146, 183].

2.3. The vertebrate host

A wide range of vertebrate species is parasitized by *I. ricinus-persulcatus* complex ticks (Fig. 1, Tab. I), but not all species are reservoir

competent. A reservoir host must be able to: (i) host vector ticks; (ii) acquire the agent from infectious ticks; (iii) allow the agent to multiply and/or develop and persist in its body; and (iv) transmit the agent back to subsequently feeding vector ticks [115].

Decades of work in North America and Eurasia have revealed the importance of small mammals such as mice (e.g. genera *Peromyscus*, *Apodemus* and *Neotoma*), voles (e.g. genera *Myodes* [*Clethrionomys*] and *Microtus*), chipmunks (e.g. *Tamias striatus* and *T. sibiricus*), and shrews (e.g. genera *Sorex*, *Neomys*, and *Blarina*) for the enzootic transmission cycles of LB spirochetes [79, 127, 154, 271, 282]. Colonial seabirds and ground-foraging birds, such as pheasants (*Phasianus colchicus*), blackbirds (*Turdus merula*), European and American robins (*Erithacus rubecula* and *T. migratorius*) were soon recognized as being efficient reservoir hosts as well [46, 96, 121, 188, 231]. Medium-size mammals such as squirrels (*Sciurus carolinensis*, *S. vulgaris*, *S. griseus*), raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), and hares (*Lepus europaeus* and *L. timidus*) are involved to varying degrees in the enzootic cycles of LB spirochetes [46, 97, 107, 129, 141, 268]. Lizards rarely are reservoir hosts for pathogenic LB spirochetes in the USA, although certain species (e.g. *Eumeces inexpectatus*) may support enzootic cycles of LB spirochetes [43, 133, 266]. Green lizards (*Lacerta viridis*), sand lizards (*L. agilis*), and common wall lizards (*Podarcis muralis*) play a role in the enzootic cycles of *B. lusitaniae* in Europe [2, 147, 156, 234].

A number of vertebrate species are considered to be barriers for LB spirochetes. These hosts (e.g. deer, certain lizard species, and livestock in certain systems) are incompetent hosts [128, 180, 269], but feed vector ticks. These species thus can reduce, or “dilute” the proportion of infected ticks by diverting infected nymphs and naïve larvae away from reservoir hosts [116, 157]. By providing blood meals to ticks, however, they nevertheless play important roles in increasing tick survivorship, the maintenance of the tick population (e.g. deer), and thus may facilitate the persistence of LB spirochetes (especially if non-systemic transmission

may occur) [176]. Conversely, if ticks are host-limited, alternative hosts that have intermediate levels of reservoir competence may “rescue”, or amplify, LB spirochetes [116]. Host species may also support different species and/or strains of LB spirochetes to varying degrees. Examples, and a likely mechanism responsible for the differences in reservoir competence, are discussed in Section 4.3.

3. REPRODUCTIVE FITNESS (R_0) AS A FRAMEWORK TO ASSESS MOLECULAR INTERACTIONS AMONG LB SPIROCHETES AND THEIR VECTOR AND VERTEBRATE HOSTS

Given the continuing increase in the number of infected ticks in endemic foci as well as an expansion in the geographic range of infected vector ticks, what are the biological characteristics, or adaptations, that help make the LB system so successful?

Successful transmission requires complex interactions among the agent, the vector and vertebrate host. It can be difficult to see the relative importance of a particular interaction among the many. Thus, it is useful to construct simplified frameworks that represent the current understanding of the system. These frameworks allow researchers to organize their knowledge of the system and thereby identify gaps – from there, multiple competing hypotheses can be constructed, guiding future research directions.

Many authors have used the parasite triangle as a simplified framework [179, 218]: the triangle’s vertices are represented by the pathogen, vector host, and vertebrate host (Fig. 1). The triangle illustrates the interdependency of the three organism types for the maintenance of the vector-borne agent and represents a community of interacting organisms – a “biocoenosis”. The parasite community triangle is inseparable from the environment in which it sits, where it is under the constant influence of other biotic as well as abiotic factors. To synthesize the dynamic outcome of the parasite dynamics (i.e. enzootic, epizootic, or fadeout), the parasite triangle is linked with another – more quantitative – framework, R_0 . R_0 is placed at the

triangle's center to represent the summation of the triangle's processes in a given ecosystem.

R_0 is the pathogen's reproductive number and is quantified in terms of numbers of infected hosts. It is unitless and is measured as the number of hosts that become infected as a result of the introduction of one infected host into a population of completely susceptible hosts [8]. R_0 therefore is a measure of the agent's reproductive fitness. If $R_0 = 1$, one secondary case results from the primary case; the pathogen exactly replaces itself and is said to be endemic in the host population. If $R_0 > 1$, many secondary cases result from the primary case, describing an epidemic. If $R_0 < 1$, then the primary case fails to infect any susceptible individuals, and the pathogen goes extinct. In the case of LB spirochetes, one can ask, "given a population of susceptible hosts and an established population of vector ticks, how many future hosts will become infected with LB spirochetes upon the introduction of one infected host?"

Randolph and Craine [219] developed the first mathematical expression for R_0 for a tick-borne pathogen, which subsequently has been further developed analytically [99] (see Tab. II for further explanation and examples of factors influencing components of R_0 , Fig. 3). R_0 is a summary of the entire parasite triangle conditional on the particular ecosystem, where processes within the parasite triangle are distilled into parameters within a mathematical expression. Therefore, R_0 provides a framework to analyze the impact of traits and/or processes (e.g. molecular, organismal, ecological, environmental) in maintaining LB spirochetes in nature. For example, β_{V-T} represents the transmission efficiency of LB spirochetes from an infected vertebrate to a naïve tick. In laboratory xenodiagnostic studies, β_{V-T} can be estimated as the proportion of naïve larvae that become infected upon feeding on an infected host. Thus, the hypothesized importance of a particular phenotype of LB spirochetes for infection can be tested by quantifying β_{V-T} and relating it to R_0 . For certain questions and depending on available data, the framework (i.e. parameters and the mathematical expression) can be modified to be more reductive or synthetical.

In the following section (Section 4), I highlight some of the biological traits and processes that enhance the transmission of LB spirochetes (i.e. favoring $R_0 \geq 1$) in each of the major components of the parasite triangle. In addition, attention is paid to processes that facilitate interactions among LB spirochetes and their hosts, including vector-vertebrate interactions. Phenotypes (with a genetic basis) of LB spirochetes that favor $R_0 \geq 1$ would be considered adaptive. Table II lists the parameters of R_0 and examples of biological traits of the pathogen, vectors, hosts, as well as the interactions (detailed in the following sections) that would increase or decrease R_0 ².

4. INTERACTIONS AMONG LB SPIROCHETES AND THEIR VECTOR AND VERTEBRATE HOSTS

To help categorize these interactions, I divide the transmission cycle of LB spirochetes into four key events: transmission between the vector and vertebrate hosts (occurring at least twice) and persistence between transmission events (occurring at least once in the vertebrate and at least once in the tick host). During the transmission events, all three organisms interact. The feeding lesion in the vertebrate host and the midgut of the tick represent the two theaters in which molecules from all three organisms perform. If transmission is successful, the drama continues for two of the three organisms; that is, the spirochete tries to persist in either the vertebrate or tick host until the next transmission event occurs. Here I focus on examples from the *B. burgdorferi*-*I. scapularis* system.

4.1. Vector competency and LB spirochete transmission from vertebrate to tick ($\beta_{T-V}^* \beta_{T-V}$)

When a susceptible tick parasitizes an infected vertebrate, what determines whether

² Phenotypes of the vector and vertebrate hosts that lead to $R_0 \geq 1$ would not be defined as adaptations of the pathogen, nor would they be considered adaptations of the vector or the host, unless they actually increased their fitness.

Table II. A model (i.e. an hypothesis) of the sequence of steps for the determination of the susceptibility of a host to infection of a given LB spirochete strain/species (one component for determination of reservoir status) and transmission success from tick to vertebrate (β_{T-V}). Susceptibility status is determined in the midgut of the feeding tick and is the outcome of interactions between the particular LB spirochete species and strain and the host species complement of the innate immune system. See text for more explanation of the tick to vertebrate transmission coefficient (β_{T-V}) [219]. The relationship between spirochete load and β_{T-V} may not be linear.

Step	<i>I. ricinus-persulcatus</i> complex nymph infected with a particular LB spirochete species or strain		
	Strain 1	Strain 2	Strain 3
(1) Host blood enters midgut of infected nymphs; spirochete population is mainly expressing OspA	Yes	Yes	Yes
(2) Can spirochetes bind host species-specific regulatory proteins of the complement pathway and avoid detection and destruction? (Outcome may vary with host species.)	Yes	Somewhat	No
(3) Spirochete status in the <u>tick midgut</u>	Multiplies profusely	Multiplies moderately	Destroyed
(4) Spirochete population begins to upregulate OspC and downregulate Osp A as they migrate from the tick midgut through hemocoel to the salivary glands, and then are transmitted to the host	Yes	Yes	None
(5) Transmission efficiency (β_{T-V}) to the host, if all else equal.	Higher spirochete inoculum contributes to greater β_{T-V}	Moderate spirochete inoculum contributes to lower β_{T-V}	No transmission ($\beta_{T-V} = 0$)

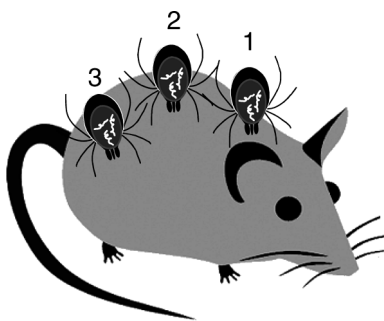


Figure 3. Three *I. ricinus-persulcatus* complex nymphs – infected with different LB spirochete species and/or strains – feeding on a reservoir host.

transmission will occur and the efficiency of transmission (i.e. what proportion of feeding vectors will become infected)? The extent

to which LB spirochetes colonize a tick, reproduce, survive in the midgut through the molt, migrate to the salivary gland and then are transmitted to a susceptible vertebrate describes how “competent” a tick is for the spirochete. The degree of vector competency is a manifestation of many molecular interactions between the pathogen and tick. Here I consider two aspects of vector competency: (i) the ability of LB spirochetes to infect a tick (β_{V-T}), and (ii) the ability of LB spirochetes to persist through the molt (β_{T-T}). I discuss the third aspect of vector competency – the ability to transmit to a vertebrate host – in Section 4.3.

Together with ticks in the *I. ricinus-persulcatus* complex, other sympatric tick species have ample opportunities to become infected with LB spirochetes. I first summarize the studies

that rule out the competency of two tick species that often share hosts with *I. scapularis*. I then turn to a brief discussion on tick innate immunity, which likely plays a large role in determining vector competence [257]. Finally I devote the rest of this section to highlighting particular *B. burgdorferi*-*I. scapularis* molecular interactions that illustrate our understanding of how *B. burgdorferi* is acquired and maintained through the molt. Any variation within the vector and LB spirochete populations for these and other traits can influence the relative fitness of various strains and therefore shape adaptations and evolution.

4.1.1. Factors influencing LB spirochete transmission from the vertebrate to the tick

As with a reservoir host, a tick is vector competent if it is able to: (a) become infected with the agent when feeding upon a competent vertebrate; (b) maintain the agent through the molt; and (c) transmit the agent to a competent host during a subsequent blood meal [115]. Before exploring the traits that contribute to the high vector competency of *I. ricinus-sensulcatus* complex vectors, it is worth examining recent research on mechanisms resulting in inefficient transmission in other tick species to improve understanding of mechanisms resulting in vector competence.

In the eastern USA, two human-biting ticks, *Amblyomma americanum* (the lonestar tick) and *Dermacentor variabilis* (the American dog tick), often overlap with *I. scapularis* in habitat, activity, and in vertebrate hostrange [23, 255]. Their prevalences of infection with LB spirochetes in the field are very low, however, and there is overwhelming laboratory evidence that both species are highly inefficient or incompetent vectors [155, 166, 201, 204, 239]. As will be explained below, different mechanisms prevent efficient vector competency in these two species.

A. americanum, the vector of the agents of human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), canine ehrlichiosis (*Ehrlichia ewingii*), and possibly the unknown agent of southern tick-associated rash illness (STARI), commonly overlaps with *I. scapularis* in south-

ern states and increasingly in northern states [3, 23, 53, 83, 155, 243]. Field collections of questing and on-host *A. americanum* rarely have been infected with *B. burgdorferi*³ [86, 273]. Laboratory transmission experiments show that *A. americanum* larvae are: (i) infected with *B. burgdorferi* very inefficiently if at all ($\beta_{V-T} \sim 0$) [155, 166, 201, 238, 239, 253]; (ii) highly inefficient at maintaining *B. burgdorferi* through the molt ($\beta_{T-T} \sim 0$) [155, 201, 239]; and (iii) incapable of transmitting the spirochete to susceptible hosts ($\beta_{T-V} = 0$) [238]. Ledin et al. [132] found that *A. americanum* saliva effectively kills *B. burgdorferi* – fewer than 13% of spirochetes that were exposed to saliva for 48 h remained alive. Thus, most spirochetes in the feeding lesion of a host do not survive to infect *A. americanum*. The borreliacidal factor(s) has (have) not yet been identified, but may be related to observed differences in the sialomes (i.e. set of mRNAs and proteins found in salivary glands) of *A. americanum* and *I. scapularis* [281].

D. variabilis, the vector for the agent of Rocky Mountain spotted fever (*Rickettsia rickettsii*), overlaps much of *I. scapularis*' range [23, 53]. In contrast to *A. americanum*, engorged *D. variabilis* removed from wild hosts and dogs have been found to harbor *B. burgdorferi* spirochetes ($\beta_{V-T} > 0$) [4, 94], and a low percentage of questing adults have been found to be infected with viable *B. burgdorferi* spirochetes, indicating that ingested spirochetes can be maintained transstadially, albeit inefficiently ($\beta_{T-T} > 0$) [204, 283]. Nevertheless, laboratory experiments have shown that *D. variabilis* nymphs that previously had fed as larvae on *B. burgdorferi*-infected hosts subsequently are unable to infect susceptible hosts ($\beta_{T-V} = 0$) [155, 166, 201, 239]. The combination of field and laboratory

³ While a few older studies do report finding *B. burgdorferi*-infected field *A. americanum*, it is possible that the ticks were infected with *B. lonestari*, a relapsing fever spirochete discovered in the late 1990s that would not have been distinguishable from *B. burgdorferi* in the diagnostic assays used by those studies [15].

data strongly indicates that *D. variabilis* is ecologically unimportant as a vector.

Laboratory studies suggest that *D. variabilis* kills spirochetes after ingestion. Johns et al. [110, 111] showed that when *B. burgdorferi* organisms were injected into the hemocoel of *D. variabilis*, there was an increase in hemocyte count and subsequent lysis and reduction of live spirochetes. These authors also identified an antimicrobial peptide, a defensin with borreliacidal properties, whose production is increased in response to *B. burgdorferi* challenge, leading to rapid spirochete lysis [112]. While *I. scapularis* hemolymph also exhibits moderate borreliacidal activity, killing seems to depend on cell-mediated processes rather than antimicrobial peptides. Johns et al. hypothesize that the cell-mediated process is much slower in *I. scapularis*, allowing spirochetes time to disseminate to tissues such as the salivary glands, where they can evade the tick's immune response. Furthermore, although a defensin also has been found in *I. scapularis*, it does not appear to have borreliacidal activity [108]. These data explain in part how *D. variabilis* can acquire *B. burgdorferi* from an infected host and maintain some spirochetes through the molt, but then cannot transmit them to reservoir hosts [253].

These studies implicate the role of innate immunity, which is much less understood for ticks than for insects, in determining the degree of vector competency [257]. Among *B. burgdorferi* vectors, competency levels vary. Compared to northern populations of *I. scapularis*, infection prevalence of questing ticks is generally lower in *I. pacificus* populations [60], and much lower in southern populations of *I. scapularis* [42, 187]. While ecological conditions seem to limit the enzootic dynamics of *B. burgdorferi* in those areas (see Section 5), genetic differences in innate immunity may also contribute to the observed variation. Some early laboratory experiments [203] showed that *I. pacificus* is less efficient at acquiring and maintaining *B. burgdorferi* spirochetes compared with northern and southern populations of *I. scapularis* [201], which were comparable. A more comprehensive comparison of similar studies is not possible because different researchers used different methods to infect

hosts, laboratory hosts, and spirochete strains. Motivated to design prophylactic therapeutics and vaccines, researchers surely will uncover more innate immune mechanisms influencing vector competence of *I. ricinus-persulcatus* complex vectors.

4.1.2. Successful colonization of vectors by *LB* spirochetes

Despite exhibiting some borreliacidal activity [111, 132], *I. scapularis* is a highly efficient vector for *B. burgdorferi*. Research in the last decade has identified several proteins expressed by *B. burgdorferi* in the tick environment that seem to be important for pathogen acquisition and persistence in the tick; these have been described in detail in several previous reviews (e.g. [10, 64, 104, 191]). Here I highlight a well-characterized molecular interaction at the *B. burgdorferi*-*I. scapularis* interface that results in a high degree of vector competence.

Several outer surface proteins have been shown to be necessary for successful infectivity of *I. scapularis* by *B. burgdorferi* (β_{V-T}). Both outer surface proteins (Osp) A and B are preferentially expressed by *B. burgdorferi* when colonizing the tick, and then are generally down-regulated during tick feeding and when in the vertebrate host [30, 50, 52]. OspA has been found to bind to a tick midgut receptor, TROSPA [192]. Experiments using antibodies against OspA as well as RNAi manipulations to inhibit expression of OspA by the spirochete or expression of TROSPA by the tick show that OspA is critical for spirochete colonization [49, 190, 192, 299]. OspA has also been found to stick to itself, and thereby may further facilitate spirochete-spirochete adherence and interaction [190]. OspB, which shares a promoter with OspA [106], also seems necessary for *B. burgdorferi* adherence to the tick midgut, although the putative midgut receptor has not been identified [63, 174, 299]. OspA and B probably help *B. burgdorferi* to remain attached to the midgut, rather than being actively removed or destroyed by the tick, during feeding and/or molting. Thus, along with the lack of a strong innate immune response against *B. burgdorferi*, expression of the TROSPA receptor contributes

to the vector competence of *I. scapularis*. Within the R_0 framework, OspA and B (and other proteins like BB0365, [194]) seem critical to increasing both the transmission coefficient from vertebrate to tick (β_{V-T}) and the transstadial transmission coefficient (β_{T-T}), and thus are considered to be adaptive traits.

With the recent availability of several LB spirochete genome sequences, high throughput molecular techniques, and sophisticated molecular manipulations in combination with transmission experiments, researchers will continue to characterize spirochete proteins and their phenotypes. Some, like OspA, will be critical for successful LB spirochete infection, persistence, and transmission; others may not be essential [177]. For example, OspD expression is highly correlated with *B. burgdorferi* replication, which occurs while the tick is feeding and digesting its meal, but ceases after the molt [263]. *OspD*-mutants have lower colonization rates in the tick midgut, which results in lower spirochete burdens but not in lowered transmission to susceptible hosts [138]. This finding of a partial effect of a protein on acquisition or maintenance of *B. burgdorferi* implies that multiple genes and gene products are acting in a compensatory manner [138, 194, 297], although it may also be that researchers have not found the context in which a particular protein plays a critical role.

4.2. LB spirochete persistence in the tick (β_{T-T})

Once *B. burgdorferi* successfully colonizes the midgut of *I. scapularis*, it must survive the midgut environment during the blood meal as well as through the molt and then persist in the midgut until the next blood meal. During the larval blood meal and just after attachment, the spirochete population within the midgut continues to increase [253]. The population then decreases either before or after the molt [32, 202]. This decrease can represent an inability to remain anchored to the midgut epithelium and/or to a lack of necessary resources such as N-acetyl-glucosamine [32, 202]. The decrease in spirochetes in flat nymphs represents a bottleneck in population size (and perhaps diversity as well). Furthermore, the spirochete burden

in a flat *I. scapularis* nymph seems to be a critical component for infection, as below a certain threshold, infected nymphs may not successfully infect vertebrate hosts [49].

Surviving spirochetes must persist in the midgut until the tick contacts another vertebrate host. In the northeastern USA, approximately 9 to 10 months elapse between the peak larval and nymphal host-seeking periods, and approximately 6 months elapse between the peak nymphal and adult host-seeking periods. Bb0690 is a *B. burgdorferi* protein that is a homolog of Dps, a DNA-binding protein known to protect DNA against damage during starvation or oxidative stress in *Escherichia coli* and other bacteria. Dps provides a critical role in preserving the integrity of *B. burgdorferi* DNA in flat ticks between blood meals. Li et al. [139] infected *I. scapularis* larvae with *Dps*-deficient *B. burgdorferi*, allowed them to molt, and then challenged naive mice with nymphs of two ages – 3 weeks and 4 months post-ecdysis. For the younger nymphs, spirochete burdens in engorging ticks were similar to those in control, infected nymphs, and transmission to mice was 100%. For the older nymphs, however, spirochete burdens plummeted and no transmission to mice occurred. Furthermore, they show that Dps expression is highest in questing ticks and lowest in feeding and engorged ticks. Consequently, they hypothesize that BB0690 is crucial for *B. burgdorferi* survivorship, and thus adaptive, during the inter-blood meal period, but not for transmission per se. Future studies likely will reveal other proteins that aid LB spirochete persistence during off-host periods of the tick's lifecycle.

4.3. LB spirochete transmission to the vertebrate host (β_{T-V})

Transmission of *B. burgdorferi* by nymphal *I. scapularis* generally requires 40 to 48 h, during which the spirochete replication in the engorging tick is followed by migration through the hemocoel and invasion of the salivary glands [103, 200] (although transmission of LB spirochetes by nymphal *I. ricinus* may occur sooner, illustrating variation among different LB systems [114]). During this time, the drama that heavily influences spirochete transmission

(β_{T-V}) occurs in the tick midgut, where the spirochetes and host factors in the blood meal interact. The quantitative nature of these interactions likely varies with the particular combination of the LB spirochete species/strain and the host species [51, 98]. While this review highlights how certain salivary molecules affect both the transmission and acquisition of LB spirochetes at the feeding lesion, and how antimicrobial peptides interactions with spirochetal factors in the midgut can influence these transmission coefficients, a more complete understanding of three-way interactions among spirochetes, the tick and the host would be a fascinating area for future research.

The many different vertebrate host species provide a myriad of ecological niches in which LB spirochetes can exist. As with other organisms, LB spirochete species vary in their degree of host specialization: some genotypes (both among and within species) are niche specialists adapted to phylogenetically closely related vertebrates (e.g. mammals or birds), while others are generalists that can exploit more than one such niche [122, 123].

The quality of a vertebrate species as a niche for LB spirochetes seems to be largely mediated by species-specific innate immunity, which comes into play as soon as the spirochetes contact complement in the host blood meal [123]. At the beginning of infection, before any pathogen-specific antibodies are made, the invading spirochetes are attacked by the non-specific alternative complement pathway. Deposition of complement components such as C3b on surfaces of invading pathogens results in opsonization and direct killing by the formation of membrane attack complexes. C3b deposition, in the alternative pathway, occurs spontaneously and indiscriminately on host and pathogen surfaces that are in contact with blood. To avoid the destruction of host cells, a number of regulatory proteins regulate C3b deposition.

Interestingly, LB spirochetes express several complement regulator-acquiring surface proteins – such as BbCRASP-1, BbCRASP-2, BbCRASP-3, OspE, and other Erp proteins (of which each spirochete may possess multiple forms). These proteins can bind regulatory proteins of the alternative complement pathway,

such as factor H and factor H-like protein 1 (FHL-1) [1, 100, 118, 119, 260, 284]. Once coated with these host regulatory proteins, spirochetes avoid recognition and eradication by complement. Thus it appears that the host range for LB spirochetes is determined by their sensitivities to complement of particular host species [260], and furthermore, the ability to bind factor H and FHL-1 appears to depend on the genotype of LB spirochetes [123].

Because complement-mediated selection operates directly in the midgut of feeding ticks, spirochetes are destroyed directly in the ticks, prior to transmission to the host. The interaction between spirochetes with complement therefore has a major impact on LB spirochete transmission dynamics and R_0 in nature. For example, it is hypothesized that the paucity of infected *I. pacificus* nymphs is due to the sensitivity of *B. burgdorferi* to complement of western fence lizard (*Sceloporus occidentalis*), and because *I. pacificus* immatures tend to feed on lizards more than sympatric rodents [128].

Antigenic switching of LB spirochetes in the tick and interactions with tick salivary proteins enable early infection of reservoir hosts (β_{T-V}).

When an infected host-seeking nymph contacts a host and begins to feed, an increase in *B. burgdorferi* replication in the midgut is accompanied by differential regulation of various genes. One oft-cited shift is the down-regulation of *ospA* and up-regulation of *ospC* in spirochetes in the midgut [148, 186, 245, 246]. While not all spirochetes undergo this transformation, there is proportionately lower *OspC* expression by spirochetes in the midgut of flat (i.e. unfed) ticks, and there is little *OspA* expression by spirochetes in the salivary glands or feeding lesion. This shift in the expression of this pair of genes has become a paradigm for the differential expression of suites of genes associated with different steps in the natural transmission cycle of LB spirochetes between the tick and vertebrate hosts. In the case of *OspC*, an abundantly expressed outersurface protein, this shift reduces the effect of the host adaptive immune response to eliminate spirochetes in the tick midgut. Because spirochetes

downregulate OspC expression in the tick mid-gut, host anti-OspC antibodies ingested by an infected tick cannot prevent transmission from the tick to the vertebrate.

OspC expression is necessary for early infection of the host during natural tick-mammal transmission [92, 262], but can have variable expression in later infection [101]. OspC aids in evading the host immune system during early infection as well as during dissemination, but a precise understanding of the mechanisms remains elusive [215]. Although OspC was found to bind to the tick salivary gland [193], *ospC*-mutants still can migrate to the salivary glands [92], but are unable to establish early infection in mammalian hosts [275]. Subsequently, it was discovered that OspC binds to the salivary protein Salp15 [216], which previously had been found to inhibit CD4⁺ T cell activation [9, 75]. Thus, binding to Salp15 may protect *B. burgdorferi* from antibody-mediated killing in the host and thereby facilitate successful early infection. Within the R₀ framework, the interaction between OspC and Salp15 may increase β_{T-V} (and even β_{V-T} if non-systemic transmission occurs) and thus be adaptive for *B. burgdorferi*. RNAi silencing of Salp15, however, does not completely inhibit spirochete transmission [216]. Thus there may be other proteins to which OspC binds or mechanisms by which OspC aids in the transmission of *B. burgdorferi*. Similarly, Xu et al. [297] showed that other lipoproteins (OspA, VlsE, OspE, and DbpA) can compensate for the lack of OspC in aiding both early infection and dissemination prior to the initiation of the adaptive immune response.

4.4. Persistence of infectious LB spirochetes in the vertebrate host (r)

Once LB spirochetes gain access to the vertebrate host, they need to survive until they are transmitted back to competent ticks. This effectively means that the recovery (r) and mortality (h) rates of the vertebrate must be low relative to the contact rate with another tick. Here “recovery” specifically refers to the host’s loss of ability to transmit spirochetes to another tick, i.e. due to immunity. The *B. burgdorferi* gen-

ome does not seem to contain known virulence genes [37, 73, 276], so it appears at this time that successful infectivity of and persistence within a mammal host depend primarily on evading the host’s immune system, rather than exploiting the host tissues for reproduction and growth [85, 216, 276]. Within the vertebrate host, the spirochetes are localized first in skin near the feeding lesion (giving rise to the diagnostic erythema migrans or “bull’s eye” rash), then travel through the bloodstream, and finally disseminate to skin and other varied organs such as joints, heart, and bladder. It is hypothesized that spirochetes persist in the extracellular matrix of such organs until transmission to another feeding tick can be attempted (Fig. 4) [236]. While this sequence of events has been thus documented, the relative importance of the roles of hematogenous and extravascular dissemination for eventual transmission of spirochetes to subsequently feeding ticks is unknown [124].

4.4.1. Early infection: evading the host’s immune system

The mechanisms underlying evasion by LB spirochetes from host defenses during early infection depend critically on functional proteins being present in adequate quantities within a crucial time window. Among these proteins is OspC, which was previously discussed in the context of “preparing” spirochetes in ticks for transmission to the vertebrate and which is critical for early infection of mammalian hosts [92, 215, 262]. Also, as mentioned earlier, even prior to infection of the host, CRASP and Erp proteins play an important role in helping LB spirochetes evade host complement-mediated killing.

4.4.2. Spirochetemia

To continue evading the host immune system, LB spirochetes eventually down-regulate OspC and introduce new antigenic actors. One of the mechanisms that help LB spirochetes cope with host adaptive immunity is antigenic variation of an outer membrane lipoprotein, expressed by the variable major

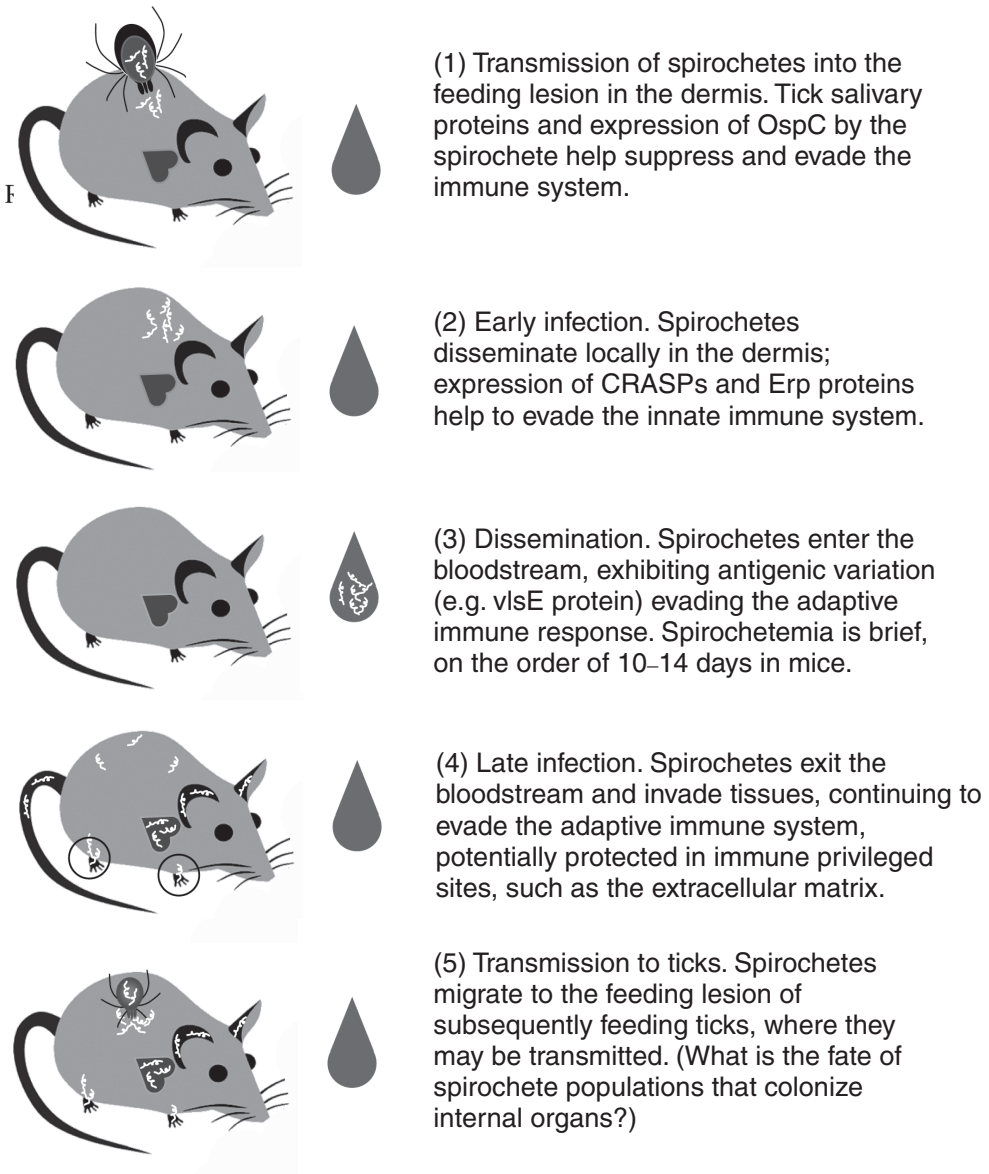


Figure 4. Infection and persistence of spirochetes in the reservoir host and transmission to subsequently feeding ticks. LB spirochete species and strains may vary in duration of persistence and infectivity (r) in a given host species [51, 98], and therefore the host-to-tick transmission coefficient ($\beta_{V,T}$) [219]. See text for further explanation.

protein-like gene (*vlsE*). The *vls* system consists of a *vls* expression site (*vlsE*) encoding a surface-exposed lipoprotein and 15 unexpressed silent *vls* cassettes. The *vlsE* protein consists

of six highly variable regions interspersed among invariable regions. In mice, segments from variable regions of the silent cassettes randomly recombine into the *vlsE* gene as soon as

four days post-infection [301]. During the infection LB spirochetes establish a large repertoire of VlsE variants that alter its antigenicity, despite the robust antibody response to conserved segments. Variation in VlsE was not observed in vitro nor in ticks, suggesting that recombination is promoted by unidentified host factors [178], such as ones participating in the host inflammatory response [160].

4.4.3. Disseminated infection and persistence

After the host adaptive immune response becomes effective (on the order of 7 to 14 days in mice and humans, but dependent on the strain or genotype [55, 286]), spirochetes disappear from the bloodstream but have disseminated to internal organs, synovial fluid, and collagenous tissue in the skin, joints, and tendons [101]. Spirochetes may find protection in these sites from the host immune system. For instance, while passive transfer of immune sera can prevent infection of naïve hosts with *B. burgdorferi*, it cannot cure previously infected hosts [16]. Spirochete populations found in different sites [17, 195], however, may vary in susceptibility to antibodies, suggesting either tissue-dependent binding by spirochetes or tissue-dependent efficacy of antibodies. Furthermore, although antibiotics are very effective at clearing infections, PCR assays have detected residual spirochetes in some mice, dogs, and humans after treatment (see later in this Section 4.4) [25, 212, 264, 265].

The extracellular matrix (ECM) may provide an immune-privileged environment for spirochetes [33, 102]. Several *B. burgdorferi* molecules have been identified that help spirochetes attach to ECM elements such as proteoglycan decorin, glycosaminoglycans, fibronectin, and host cells ([33], and references therein). Empirical manipulations, such as antibodies against Dbp A and B, can reduce the infectivity of *B. burgdorferi* for various tissues and spirochete loads, but as with other processes, *B. burgdorferi* seems to have redundant adhesive interactions in place to ensure persistence in the ECM [17, 33, 249]. For example, Pal et al. [195] showed that BmpA and B are two spirochete lipoproteins that are

upregulated by spirochetes in joint tissue, required for colonization of joint tissues, and contribute to arthritis in mice.

LB spirochetes' interactions with certain other host cells may further help evade the immune system either intracellularly or extracellularly. *B. burgdorferi* has been found intracellularly in human endothelial cells, macrophages, and cardiac myocytes [144, 162, 189]. Extracellularly, *B. burgdorferi* can bind to the invaginations of cell membranes of fibrocytes, thereby avoiding phagocytosis as well as antibodies and antibiotics [77, 88, 175]. Future studies undoubtedly will uncover other spirochete-host cell interactions, as well as their relative importance for *B. burgdorferi* dissemination and persistence in the vertebrate host.

4.4.4. Prelude to transmission to ticks: migration of spirochetes to feeding lesion

The successful migration of spirochetes from within the vertebrate host into the feeding tick is not well understood. Presumably aggregation of spirochetes around a tick feeding lesion results in increased transmission efficiency (β_{V-T}) of the pathogen from host to ticks. Chemotaxis probably helps direct LB spirochetes towards feeding ticks [38, 142]. Salivary gland extracts of fed ticks act in vitro as an attractant for *B. burgdorferi* [250].

Narasimhan et al. [171] provide an in vivo example illustrating how the spirochetes might use vector molecules to increase the success of transmission from host to ticks. The tick salivary protein Salp25D helps detoxify reactive oxygen species produced by neutrophils at the feeding lesion, thereby increasing *B. burgdorferi* survivorship as spirochetes run the gauntlet from the mammal to the tick midgut. When Salp25D is silenced and/or when the mouse host is immunized against Salp25D, significantly fewer spirochetes colonize midguts of feeding larvae compared with controls (reducing β_{V-T}) [171]. The authors did not report the subsequent transstadial transmission coefficient (β_{T-T}), but if the proportion of infected molted nymphal ticks were lower than that of controls, it would further confirm a positive effect of Salp25D on spirochete fitness.

An emerging paradigm is the potential role of hormone molecules that can signal the presence of feeding ticks to the spirochete. Scheckelhoff et al. have found that binding by *B. burgdorferi* to the human catecholamines epinephrine and norepinephrine results in increased expression of OspA (predominantly expressed in ticks and not vertebrates) [242]. Furthermore, when researchers administered an inhibitor of catecholamine receptors to infected mice, a lower proportion of feeding ticks became infected compared to ticks feeding on control infected mice. Catecholamines also can be found in tick saliva [242]. Thus, whether it is a signal of stress by the host due to infestation with a tick, or a salivary protein signaling the presence of a feeding tick, hormones may aid in transmission of spirochetes to vector ticks.

It would appear that spirochetes persisting in the skin have a greater probability of being transmitted to ticks, given the average duration of tick engorgement (e.g. 3 to 5 days for larval *I. scapularis* on mice). Thus interactions that allow spirochetes to evade the host immune system, recolonize skin, and navigate through the ECM can be inferred to be adaptive (e.g. [164]). What are the fates, however, of spirochetes that persist in the ECM of internal organs and tissues (e.g. joints, ligaments, and synovial fluid)? Evolutionarily, these populations of spirochetes will be dead ends if they are unable to migrate to tick-feeding sites and infect hosts. An intriguing, recent study suggests that spirochetes persisting in internal organs (e.g. heart-base and tibiotarsus) of laboratory C3H mice are capable of transmission to xenodiagnostic larvae [102]. In that study C3H mice had been previously infected by needle inoculation and then treated with antibiotics 1 or 3 months post-infection. Spirochetes were not detectable or culturable at any sites, including three different skin sites, and were PCR-positive only in internal organs. Xenodiagnostic larvae were allowed to molt, and as nymphal ticks, they were able to infect C3H *scid* (i.e. severe combined immunodeficiency) mice. Prior to this study, it had been thought that these spirochetes, which were detected in joints and various other organs by PCR, were unlikely to be

infectious because they were unculturable [25]. The inference from this recent study is that the transmitted spirochetes were not residents of skin, but from persistent populations in internal organs. Further understanding the impact of these transmittable, but unculturable, spirochetes on natural transmission cycles or disease requires investigating whether these spirochetes can infect immune competent mice.

If these persistent spirochetes are rarely transmitted to immune competent hosts and therefore rarely contribute to the reproductive population of spirochetes, the “adaptive” value of the interactions is questionable (although potentially important for understanding the etiology of disease). Furthermore, an hypothesis would be that these interactions are the by-products of adaptations that help *B. burgdorferi* populations in the skin become transmitted to a tick, but which cause some subpopulations of spirochetes to locate to “dead-end” tissues.

4.5. Vector-host interactions and effects on LB spirochete transmission

Broadly speaking, vertebrate host-tick interactions that influence LB spirochete populations include the processes whereby ticks (1) contact hosts and (2) successfully obtaining replete blood meals from hosts. Both processes influence tick fitness and tick population structure such that adaptations for increasing tick feeding success should benefit both tick and spirochete populations, with the caveat that some vertebrate host species (e.g. white-tailed deer) are suitable hosts for ticks, but incompetent, or barrier, hosts for LB spirochetes. Here I will discuss the on-host interactions between ticks and hosts, focusing at the sub-tick and sub-host level and reserving discussion of the ecological factors for a later section (Section 5). These tick-host interactions may enhance the transmission coefficients (both β_{T-V} and β_{V-T}).

There have been many review articles [29, 288] written about tick-host interactions, as well as tick-host-pathogen interactions, as researchers have come to recognize the importance of the former on pathogen transmission [179, 218, 289, 290]. While researchers noted decades ago the benefits of tick salivary gland

extracts on tick feeding success and pathogen transmission [18], advances in modern molecular, genomic and proteomic tools have increased our ability to “see”, characterize, and conduct experiments to infer the functions of specific salivary proteins. It is beyond the scope of this review to catalog all the different salivary genes, transcripts, and proteins that may play an important role in enhancing LB spirochete transmission either directly or indirectly. Here, I highlight a few of the interactions to illustrate how they may influence survivorship, transmission, and fitness of LB spirochetes and refer the readers to the several excellent recent reviews and articles on the topic [29, 104, 179, 229]. An important point that previous authors have made is that just as the vertebrate host’s responses have built-in redundancy, so do the counteractive responses of the tick [226, 229].

4.5.1. The pharmacopeia of the feeding lesion: enhanced feeding success

Just as a tick has gross morphological adaptations to help it attach to a host, it also secretes microscale adaptations in the form of salivary proteins to secure a replete blood meal. The invasive injury to the integrity of the host’s tissues and blood vessels at the feeding lesion alerts the host’s repair and immune systems and triggers a cascade of processes that threaten to terminate feeding and thereby reduce the tick’s survivorship and the fitness. All else equal, ticks possessing salivary proteins that can help them evade, mitigate, or modulate host responses will feed more successfully and produce more progeny. Thus, there is strong evolutionary pressure for the selection of “countermeasure” salivary proteins.

To maintain hemostasis, the host responds by vasoconstriction, platelet aggregation, and coagulation. Maritz-Olivier et al. [151] provide a recent review on tick anti-hemostatics and highlight how these factors may be potential targets for anti-tick vaccines and therapeutics. Ribeiro et al. [226] first identified the presence of platelet aggregation inhibition factors, such as an apyrase, and noted an abundance of prostaglandin (PGE₂), a vasodilating effector,

in *I. scapularis* saliva. Two proteins that *I. scapularis* produces that prevent coagulation are ixolaris and penthalaris, which are both tissue factor pathway inhibitors [69, 71, 161]. Additionally, ixolaris recently was found to have anti-thrombotic properties [172]. Homologous transcripts for both of these proteins have been found in the salivary gland of *I. pacificus* as well [72]. Another anticoagulant, Salp14, inhibits the intrinsic pathway of coagulation [169, 170]. Finally, MP1, a fibrin(ogen)olytic agent that aids in dissolving bloodclots, has been identified [70]. Inhibition of bloodclotting may be beneficial within the tick midgut as well as in the feeding lesion.

Processes in the hemostasis response also lead to and/or interact with inflammatory processes, resulting in redness, heat, swelling, and ultimately, pain. Pain and itchiness may evoke grooming behavior, unless the tick is able to reduce the inflammation response. *I. scapularis* possesses a salivary metallo dipeptidyl carboxypeptidase [226, 228] that inactivates bradykinin, a molecule in the hemostasis pathway that also can elicit a pain response. *I. scapularis* also has a number of proteins that inhibit the alternative complement pathway and prevent the formation of anaphylatoxins, thereby reducing both inflammatory and immunological responses. In particular, Isac [279, 281] belongs to a family of anti-complement proteins [230] that have been shown experimentally to enable successful feeding [253].

Given the relatively long duration of feeding required to obtain a replete blood meal, a tick is highly susceptible to host immunological responses, particularly if the host previously had been fed upon by other ticks. Acquired immunological resistance to ticks manifests as a longer feeding duration to complete engorgement, lower engorgement rate, and decreased egg production (for adult females), and therefore, lower survivorship probabilities and/or fitness for the tick. Both the adaptive immune response (i.e. antibodies to salivary proteins) and the alternative pathway of complement activation are involved with acquired resistance [29].

I. scapularis possesses a diversity of salivary proteins and mechanisms to combat the host

immune system, and our knowledge of these proteins continues to grow. Ribeiro et al. [226] demonstrated that *I. scapularis* saliva inhibited T cell proliferation, and researchers subsequently identified an IL-2 binding protein [80, 280] as the putative mechanism. Inactivation of T cell proliferation affects both the humoral and cellular immune responses. Salp15 inhibits CD4+ T cells activation as well as IL-2 production [9], which can down-regulate Th1 response and therefore macrophage activity. Subsequently, it has been learned that Salp15 also inhibits pro-inflammatory cytokines (IL-12p70, IL-6, and TNF- β) production by binding to a lectin, DC-SIGN, on dendritic cells [105]. This finding is significant as dendritic cells, which are already present and common at feeding lesions, play a central role in initiating these adaptive immune responses.

4.5.2. *The pharmacopeia of the feeding lesion: enhanced LB spirochete transmission*

Acquired resistance to feeding ticks can reduce pathogen transmission in both directions (i.e. both β_{T-V} and β_{V-T}). Nazario et al. [173] showed how repeated infestations of guinea pigs with uninfected *I. scapularis* ticks prevented transmission of *B. burgdorferi* from infected ticks feeding subsequently. This effect is not surprising – due to a lack of co-evolution, the tick and the unnatural host species have not fine-tuned their countermeasures. In nature, however, there exists a spectrum of interactions among various host species and ticks, representing different genetic, ecological and coevolutionary histories. Some hosts, like *P. leucopus* mice in North America, can produce consistently infected ticks among multiple feedings [48] and even enhanced transmission in the case of *A. flavicollis* mice in Europe [78]. Other natural hosts, like unnatural laboratory hosts, however, develop acquired resistance, leading to reduced pathogen transmission. Davidar et al. [48] show that meadow voles (*Microtus pennsylvanicus*), although reservoir competent for *B. burgdorferi*, mount anti-tick immune responses to repeated tick infestations, resulting in reduced transmission. Acquired resistance in meadow voles resulted in larvae with lower

engorgement weights and lower molting success compared to larvae that fed on previously infested white-footed mice. Similar trends were found between European voles and mice [54, 78, 120].

Other experiments with unnatural hosts have revealed the impacts that tick saliva can exert on pathogen transmission. Ribeiro et al. [227] showed that *I. scapularis* saliva could inhibit rat neutrophil function, and in the process also prevent *B. burgdorferi* from phagocytosis. Zeidner et al. [300] demonstrated in laboratory mice that inhibition of host cytokines by *I. scapularis* saliva is critical for *B. burgdorferi* transmission. Intuitively it makes sense that the anti-haemostatic, anti-inflammatory, and the immunosuppressive properties of tick saliva would also promote pathogen transmission. As mentioned previously, Salp25D is an antioxidant that critically enables *B. burgdorferi* acquisition by detoxifying the reactive oxygen species that is part of the host's immune response to tick feeding [171]. It is in this regard that Wikel [289] writes, “the tick is clearly not just a crawling hypodermic needle and syringe with regard to the transmission of tick-borne pathogens”.

Nuttall and Labuda [179] have coined the term “saliva-activated transmission” (SAT) to describe the enhanced transmission of pathogens at the tick feeding site. SAT can help facilitate transmission of pathogens from an infected feeding tick to another uninfected tick even in a host whose serum normally may neutralize LB spirochetes (i.e. is considered an incompetent host) or that has mounted antibodies against the pathogen. Non-systematic, or co-feeding, transmission via SAT has been demonstrated as the main route of transmission by which tick-borne encephalitis virus (vectored by *I. ricinus* and *I. persulcatus*) is maintained in nature [220, 221].

To recapitulate an earlier example [216], direct use of a salivary protein by *B. burgdorferi* may enhance its transmission as a benefit of down-regulation of the host immune system by that protein. Of particular note, Salp15 expression is enhanced in infected ticks compared to uninfected ticks, suggesting that the presence of *B. burgdorferi* modifies gene

expression of *I. scapularis*. Ramamoorthi et al. [216] note that while the increased expression of Salp15 increases survival of *B. burgdorferi*, it also may increase that of *I. scapularis*, as immune responses raised against the spirochete may have negative consequences for successful feeding of the tick as well. Thus increased expression of Salp15 may be adaptive for *I. scapularis*, but only when in the presence of *B. burgdorferi*. This interaction is an example of coevolution between the microbe and vector that changes the tick-to-host transmission coefficient (β_{T-V}). These results explain, in part, past unsuccessful attempts to infect laboratory animals by needle inoculation and again illustrate how ticks are not just “walking syringes” [218, 289].

5. EVOLUTIONARY AND ECOLOGICAL CONTEXT FOR ADAPTATIONS OF LB SPIROCHETES

In previous sections, I described molecular processes through which the spirochete, tick, and vertebrate interact. The implication was that if all the intricate molecular interactions were to favor the spirochete, it would have a $R_0 \geq 1$ and be more likely to persist in future generations of vertebrate hosts and ticks. There was no explicit mention, however, of the population-level aspects of those interactions. Here, I highlight life history constraints, ecological interactions, and environmental factors that may influence the molecular interactions and thereby shape the fitness and population biology of LB spirochetes. I begin by reviewing one characteristic of the LB biocoenosis that may be the most important ecological factor contributing to the explosive emergence of LB in the USA. I then turn to other factors important not just to this parasite triangle, but to that of other vector-borne diseases as well.

5.1. Generalists all around: implications for spread and impact on LB spirochete diversity

The recent invasions of the LB tick and pathogen in the USA have been attributed

largely to anthropogenic changes that favor the ecology of the LB system and have brought it into people’s backyards and the woodlands where they recreate [14, 258]. Specifically, human-induced changes have increased the abundance of hosts for both ticks and *B. burgdorferi* and have brought wildlife and humans into closer contact. The generalist natures of the vector, the spirochete, and important vertebrate hosts subsequently have enabled the spread of LB.

Deer play a primary role in the persistence of *B. burgdorferi* in nature because they are the main determinate host on which adult *I. scapularis* feed and mate [292]. The growth and spread of *I. scapularis* tick populations are inextricably linked with that of deer. Deer populations in much of the eastern USA were nearly extirpated by the early 1900s due to uncontrolled hunting, but then steadily rose in the twentieth century due to management. In the northeastern USA, the large-scale conversion of agricultural land to secondary succession forests fuelled the increase and expansion of deer populations from coastal refugia as well as that of other woodland creatures, many of which are reservoir species. Furthermore, the conversion of agricultural land and woodlands to suburban developments in the landscape created new deer habitat close to woodlands, replete with tasty ornamentals [14, 258].

Larval and nymphal *I. scapularis* feed on deer but also on numerous other species of mammals, birds, and lizards ([5] and references therein). Lawrie et al. [130] show that *I. ricinus* exhibits anti-complement activity against a range of mammalian and avian hosts, whereas the European *I. hexagonus* (a rather host-specific tick) has a much narrower range. Thus, evasion of the host innate immune system may play a role not only in pathogen permissiveness, but in host selection as well [179]. Adaptations for a generalist feeding strategy increase the probability of securing a blood meal among the various forest habitats in which *I. scapularis* apparently can survive. While the quality of the blood meal with respect to survivorship and development of *I. scapularis* varies among species, its generalist nature contributes to its ability to invade novel ecological

environments because it is not host-limited. (The same is undoubtedly true for *I. ricinus* and *I. persulcatus*.)

While the full significance is yet unknown, it is interesting to note that in the USA, it is *B. burgdorferi*, a generalist LB spirochete species, that is spreading with *I. scapularis*. Many *B. burgdorferi* strains have been detected in several host taxa, and Hanincova et al. [97] hypothesize that it is the generalist nature of the bacterium that has enabled its contemporaneous population growth and geographic spread with that of the generalist vector [125]. Indeed, in the USA, where the LB system is dynamic, at least at the edges of its range, the generalist strategy of the tick further selects for generalist strains of *B. burgdorferi*. The reproductive success (i.e. R_0) of various strains, which may have different consequences for disease in humans [55, 286, 294], the linkages among the ecology and evolution of *B. burgdorferi* and the disease and epidemiology of Lyme borreliosis.

Within endemic foci, the LB system and the ecosystem in which it exists may be relatively stable; and it is possible that vertebrate host specialization may occur over evolutionary time. This scenario may describe the historical situation in Europe, where several more host-specialized LB spirochete species coexist that exhibit differential susceptibilities to host species-specific complement [122]. *B. garinii* and *B. valaisiana* appear to be avian-adapted [96]; *B. afzelii* tends to be mammal-adapted [95]; and *B. lusitanae* may be lizard-adapted [147, 234]. In other words, for a given vertebrate host species, each of these species may have a higher R_0 compared with *B. burgdorferi*. Indeed, researchers found that *B. afzelii* had greater fitness correlates (as quantified by β_{T-V} and β_{V-T}) than *B. burgdorferi* when both were experimentally transmitted to rodent hosts, although the generality of the findings among host species remains to be tested [233]. Some researchers [28] propose that host specialization of *B. burgdorferi* strains in LB endemic areas of the northeastern USA also can be inferred, even if it is not as delineated as in Europe. With respect to geographic structure, this pattern of specialization can result in differential migration rates of the pathogen, resulting in, for

example, a larger distribution for *B. garinii* compared to *B. afzelii* [252].

Evolutionary theories generally predict that ecological specialists should be favored (i.e. have higher R_0) over generalists if resources are abundant and dependable [293]. Specialization can occur if adaptations to different habitats are antagonistic and thus result in functional trade-offs that limit the generalist's fitness in any one habitat [137]. However, the specialist strategy may carry a fitness cost, because the vectors are generalist ectoparasites that take few blood meals. In particular, specialization can have a negative effect on the tick-to-host transmission coefficient (β_{T-V}) because the transmission of specialized genotypes, such as the rodent-adapted *B. afzelii* or the avian-adapted *B. valaisiana*, will be precluded if ticks feed on non-permissive species.

How might host-specialization of LB spirochetes arise? At least two, non-mutually exclusive scenarios could lead to host specialization of *B. burgdorferi*, assuming that adaptations to various host species do not incur a tradeoff in fitness with any other aspect of the spirochete's life history. The first scenario is purely ecological, in that hosts that consistently present more frequent opportunities for feeding ticks will thereby act to select for certain strains. If host communities (species diversity and relative abundance) are relatively constant over time, host specialization of strains can be reinforced and increasing host specialization may occur, especially for hosts that contribute proportionately more to feeding and infecting ticks. If host communities are not stable (e.g. population cycling of voles or frequently disturbed communities), selection for more generalist strains may occur.

Another hypothesized mechanism by which host-specialization of spirochetes may occur is through subpopulations of ticks that vary genetically in host preference or feeding success. These tick populations would co-exist by feeding on different subsets of the vertebrate community as immatures, giving the overall appearance of a generalist strategy. This ecology may allow for increased host specialization of LB spirochete strains, as is seen within the *I. uriae*-driven system in

which *B. garinii* cycles among sea bird species [158, 159, 188]. The convergence of adults on deer (or other larger mammals), however, may pose a challenge to this hypothesis if mate selection in LB vectors is random rather than assortative.

In addition to experiencing selection by host innate immune systems, the population structure of LB spirochetes may also be sculpted by host adaptive immunity. It has been hypothesized that adaptive immunity targeting ospC or antigens at linked loci play an important role in the population biology of LB spirochetes, through imposing negative frequency-dependent selection [24, 125, 213]. Theory predicts that the fitness of a common spirochete genotype relative to rare spirochete genotype will decrease within the host population as the number of immune hosts increase. Rare spirochete genotypes, therefore, will enjoy a fitness advantage that will then decline as they become more common. Frequency-dependent changes in fitness should result in quantitative changes in spirochete population structure over time and help maintain spirochete genetic variation. Indeed, significant year-to-year differences have been observed in the frequency distributions of *B. burgdorferi* genotypes in host-seeking ticks collected in the northeastern USA [213].

At this time, the role of host adaptive immunity on the observed fitness variation among strains is unknown. In particular, if expression of antigenic proteins varies between the tick and vertebrate environment, the selective effect of adaptive immunity may be somewhat damped, depending on the degree of cross-protection of antibodies to similar antigens (e.g. ospC types). The impact of frequency-dependent selection also depends other factors, such as the relative use/abundance of various host species by vectors, the relative roles of innate and adaptive immunity, the longevity of the immune responses, as well as the longevity of the hosts themselves. Other non-host related factors selecting on unrelated traits may also indirectly influence the observed fitness variation among strains and thus the population structure of LB spirochetes.

5.2. Abiotic factors and seasonality of host-seeking

The seasonal activity of non-nidicolous hard ticks refers to their host-seeking activity, during which they climb atop vegetation and wait for a passing vertebrate host. As they host-seek, or “quest”, ticks are vulnerable to a number of environmental factors, most importantly desiccation. To avoid unfavorable environmental conditions (e.g. cold winters or hot summers) ticks host-seek only during certain periods of the year [256]. The initiation and termination of seasonal host-seeking activity seem to be photoperiod-dependent and vary among tick species and with climate [19, 66, 90, 126, 256].

The shape of seasonal activity (= phenology) curves (Fig. 2) reflects a summation of factors that trigger the onset of host-seeking, the nutritional state of the ticks, and factors that remove them from the population of questing ticks. Ticks can host-seek while their energy reserves are sufficient [222]. The decline in the questing tick population over time is due to abiotic factors affecting questing behavior, mortality, as well as contacting vertebrate hosts [223, 224].

Different life stages of ticks can be active at different or similar times of the year. For example, in the northeastern USA the peak of seasonal activity of *I. scapularis* nymphs precedes that of larvae by at least one month, whereas there is much more overlap in the Upper Midwest [53]. In contrast, in Central Europe, seasonal activities of all life stages of *I. ricinus* are more synchronized with two general peaks of activity – one in spring and another in late summer/early autumn [90].

The degree of synchrony among life stages (given overlap in host species use) can influence the R_0 of LB spirochetes. This is because the duration of infection among the host community is dynamic throughout the year. For example, as noted above, in the northeastern USA, *P. leucopus*, a relatively short-lived animal, plays an important role in the transmission cycles of *B. burgdorferi*. For *P. leucopus* to contribute to *B. burgdorferi* persistence in nature, *P. leucopus* must survive from when they are first infected by nymphs (late spring) until larvae emerge

(late summer). As a result, the degree of synchrony of nymphal and larval host-seeking, which is determined by abiotic factors, can affect the efficiency of vertebrate-to-tick transmission (β_{V-T}) and therefore R_0 [125, 181, 185].

In addition to surviving, *P. leucopus* and other reservoir hosts must remain infectious until larvae emerge for the transmission cycle to be completed. In the laboratory, *P. leucopus* generally has been found to be infectious to xenodiagnostic larvae for much longer than their average natural lifespan [57] (although see [140] for an exception). Thus the traditional view holds that the infectivity of LB spirochetes to ticks lasts lifelong in reservoir hosts, and therefore, as long as hosts survive long enough, transmission to the next cohort of ticks will occur. Recently, however, it has been experimentally demonstrated that the duration of infectivity (r) can vary among strains, and for some strains, may decline significantly within a few weeks post-infection [51, 98]. The intensity of vertebrate-to-tick transmission (β_{V-T}) therefore can vary considerably among spirochete strains in *P. leucopus*, and perhaps with different strain-host species combinations. The variation in duration of infectivity may be a function of the ability of the strain to evade the vertebrate host's immune system, persist in the host, and remain transmissible to ticks.

In ecosystems with asynchronous feeding of nymphs and larvae, all else equal, natural selection will favor long-lived strains that are persistently infectious [185]. In other words, long-lived strains will tend to have higher R_0 than short-lived strains, and over time, short-lived strains will go extinct unless other factors allow them to persist. Indeed, in questing nymphs collected in *P. leucopus* habitats of northeastern USA, where there is less synchrony, the infection prevalence of a *B. burgdorferi* genotype with short-term infectivity in *P. leucopus* (*ospC*-E = IGS9, an RST 3 type) is much lower than that of a genotype with long-term infectivity (*ospC*-A = IGS1, an RST 1 type) in the same host [27, 97, 287]. In contrast, in ecosystems with greater synchrony, short-lived strains might not be as disadvantaged. Furthermore, if there were tradeoffs in adaptations between transmission and persistence,

short-lived strains may even possess a fitness advantage in areas with synchronous feeding [181, 185]. These predictions are supported generally in a recent large-scale survey of questing *I. scapularis* nymphs in the USA: *B. burgdorferi* RST 1 strains are enriched in the Northeast, whereas RST2/3 types predominate in the Midwest [76]. It remains to be investigated how factors other than tick phenology (e.g. host communities and tick genetics) also may impact this difference in distribution of strains.

5.3. Aggregation of ticks on hosts and non-systemic transmission

In many host-parasite systems, parasites are distributed among hosts in an aggregated, rather than a random, manner [248]. Various hypotheses have been posed to explain the evolution of aggregated parasite distributions [6, 7], as well as how they result from heterogeneities in spatial distributions of hosts and parasites, behavior, immune status, etc. [291]. For vector-borne pathogens, aggregation of vectors on hosts obviously is important for pathogen maintenance, but the overall importance for LB spirochete persistence in nature is still undetermined.

Aggregation of ticks on hosts can increase vector survivorship and transmission efficiency, both of which may potentially increase R_0 . Increased numbers of co-feeding ticks result in greater repression of the host immune response due to proteins in tick saliva that have suppressive properties [48, 184], resulting in increased engorgement weights and feeding success [78, 134]. By increasing tick survivorship, the ratio of ticks to hosts (N/H) increases, thereby increasing R_0 . By helping ticks feed to repletion, aggregation may increase LB spirochete transmission from host to larvae (β_{V-T}). In both USA and European studies [78, 134], there was a positive density-dependent effect on transmission rates of LB spirochetes from infected mice, but not from infected voles. Aggregation similarly should increase the probability of transmission from an infected nymph to a host (β_{T-V}). It is unclear, however, how aggregation would affect R_0 – both when co-feeding nymphs are infected with the same or different

strains/species of LB spirochetes – in comparison to a random distribution.

Simultaneous aggregation of ticks on hosts may increase R_0 through non-systemic transmission; unlike for TBE virus, however, the importance of this pathway for LB spirochete maintenance is much less certain. Among the three post-embryonic life stages, larval ticks show the greatest level of aggregation. Thus, if larval ticks co-feed with infected adults and/or with larvae infected through transovarial transmission, aggregation may increase R_0 . Similarly, in areas where there is synchronous questing of nymphal and larval ticks, non-systemic transmission could increase R_0 during parts of the host-seeking season when fewer hosts are systemically infected. Non-systemic transmission does not require competent hosts, so it may increase R_0 quantitatively as well as support less generalist strains – thereby contributing to maintenance of LB spirochete diversity. While the contribution of non-systemic transmission may be negligible or masked in systems that are not host-limited, this transmission route may be important for LB spirochete persistence in areas where reservoir hosts are not reliably abundant. For instance, non-systemic transmission permits the maintenance of endemic cycles of LB spirochetes in sheep in upland moorland habitats of United Kingdom, where alternative reservoir hosts are virtually absent [180, 222]. The features that facilitate non-systemic transmission are: (i) the immunomodulatory properties of tick saliva; (ii) aggregated distributions of ticks on the host population; and (iii) synchronized seasonal activity of different tick life stages.

In areas where there is asynchronous questing of nymphs and larvae, persistence of LB spirochetes depends on low recovery rate (r) of hosts (i.e. selection of strains with high persistence) as well as the shared use by infectious nymphs and naïve larvae of the same individual hosts in the host community. Thus any mechanism that would increase the aggregation of naïve larval ticks on reservoir hosts that were previously parasitized by infected nymphs likely would increase R_0 and LB spirochete persistence in nature.

5.4. Interactions with other pathogens and tick endosymbionts

I. ricinus-persulcatus complex ticks are infected with several microbial species, and it is unknown how they have shaped the population biology of LB spirochetes [267]. These other pathogens include viruses (e.g. tick-borne encephalitis fever virus), piroplasms (*Babesia microti* and *Ba. divergens*), and other bacteria (e.g. *Anaplasma phagocytophilum*). *I. ricinus-persulcatus* complex ticks also can vector many other pathogenic bacteria including *Rickettsia helvetica* [68, 196] and *Bartonella* spp. [22, 45], but the epidemiological and enzootic importance of these microbes is yet to be determined.

Researchers have been interested in coinfection of ticks because of the potentially complicating effects for diagnosis and treatment [267]. Coinfections mainly have been observed for the pathogens that have higher individual prevalences. Thus, the coinfections that are most often observed in ticks are between LB spirochetes and *A. phagocytophilum*. Comparisons of single infection and coinfection prevalences among the various vector species, however, suggest that the two pathogens have independent transmission cycles [135, 241, 259, 267]. This inference of independent transmission cycles was supported by Levin and Fish [136] in their experimental investigation of the effect of coinfection on both the acquisition and transmission of *B. burgdorferi* and *A. phagocytophilum* in *I. scapularis* and *P. leucopus*. They found that infection with one pathogen did not hinder the acquisition of the other pathogen (i.e. β_{T-V}), and that co-infection did not affect the transmission efficiency of either pathogen to a susceptible tick (i.e. β_{V-T}). A recent analysis of infection data in the literature similarly suggests a lack of a strong effect of other LB spirochetes and *A. phagocytophilum* on spirochete transmission dynamics [82].

Besides the better known pathogens, researchers have found other microbes of unknown pathogenicity in *I. ricinus-persulcatus* complex ticks, e.g. *B. miyamotoi*, or a *B. miyamotoi*-like spirochete, more closely-related to

relapsing fever spirochetes found in soft ticks [167, 232, 247]. As with other arthropods, rickettsial endosymbionts have been found [20, 163, 208, 240, 259] and potentially may have important effects on transmission cycles [145]. Interestingly, Steiner et al. [259] found that co-infections of LB spirochetes with a spotted fever group rickettsia endosymbiont in male *I. scapularis* ticks occurred less often than predicted based on independent transmission expectations, although Moreno et al. [163] did not observe that among their samples. Further investigations of the microbial community ecology within *I. ricinus-persulcatus* complex ticks and their hosts may improve our understanding of the enzootiology of LB spirochetes and other pathogens [44].

6. DISCUSSION: INCORPORATING AN ECOLOGICAL AND EVOLUTIONARY PERSPECTIVE INTO LB RESEARCH, MANAGEMENT AND CONTROL

This review has highlighted many putative adaptations of LB spirochetes to their tick and vertebrate hosts, as well as biological traits of the vector and host that contribute to the maintenance of the LB biocoenosis. Increases in suitable habitat, the generalist natures of the ticks, spirochetes, and important vertebrate hosts, as well as the tick's high fecundity have enabled LB spirochete populations to spread rapidly in the USA and other areas of the world. How do these characteristics affect research concerning diagnosis, disease, vaccines and therapeutics as well as efficacy of LB management and control in the landscape?

The driving goal that motivates much of the current research to understand the adaptations of LB spirochetes for vectorial transmission is the quest for anti-pathogen and/or anti-tick vaccines. What gene target(s) would be the most effective for preventing transmission to humans or companion animals, or for breaking transmission cycles in nature by vaccinating wildlife? Can we predict the likelihood of escape mutants, and are some targets more prone to produce escape mutants? Will host-targeted vaccines be more likely to select for

vaccine-resistant strains compared to human vaccines, since humans are dead-end hosts for LB spirochetes and wildlife feed many more ticks than do humans? Can we predict consequences of escape mutants on disease manifestations for humans?

An approach that the US Department of Agriculture and Centers for Disease Control and Prevention have recently investigated for reducing LB risk in the USA is the use of host-targeted acaricides targeted at deer (to reduce the tick population) [211] and small rodent reservoirs (to divert and reduce immature ticks that are likely to become infected), respectively [56]. How will vector control affect both the abundance and local strain structure of LB spirochetes? For example, by targeting rodents with acaricide for killing larvae and nymphs, the approach selects for ticks and species (and/or strains) of LB spirochetes that feed on non-rodents – furthermore, what are the implications for disease as a consequence?

Climate change is predicted to expand the distribution of *I. ricinus-persulcatus* complex ticks and LB spirochetes into more northern regions [182]. If climate change affects the seasonality of the host-seeking phenologies, how will the strain structure and disease manifestations of LB spirochetes change regionally [185]? Climate change may also shift distributions of hosts. In the upper midwestern USA, researchers have documented a northern expansion of mammal species ranges, including that of *P. leucopus*, where it has displaced many populations of the more northern *P. maniculatus* [168]. If host community composition and/or biodiversity changes as a function of landscape changes, climate change, or management, how will the strain structure and disease manifestations of LB spirochetes change?

Predicting the impact of environmental changes and management tools, such as vaccination or vector control, on LB risk and disease manifestations in humans requires a better understanding of the ecological and evolutionary processes that shape the population biology of LB spirochetes. A little more than a decade ago, researchers learned that transmission experiments using needle inoculations can lead to bizarre or incorrect inferences about

transmission biology [218]. Today, as researchers become better able to investigate and manipulate molecular interactions in the parasite triangle, it is very important to consider the ecological and evolutionary context of the LB system to be more certain that research questions, experimental designs, and new control methods to reduce disease risk are meaningful, effective, and sustainable. Furthermore, with respect to a given spirochetal trait, researchers may find R_0 useful as a framework to assess its adaptive value for the LB bacterium and therefore its potential as a target for control.

Acknowledgements. I gratefully acknowledge contributions by Klara Hanincova and Graham Hickling (who also assisted with figures) toward earlier drafts of this manuscript. I also thank Lorenza Beati, Olaf Kahl, Nicholas Ogden, Joseph Piesman, and two anonymous reviewers for providing many helpful comments and suggestions that have greatly improved the text. I also wish to recognize Klaus Kurtenbach, whose innovative work will continue to shape our understanding of LB spirochete ecology and population biology.

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Appendix

R₀: A FRAMEWORK TO ASSESS ADAPTIVE STRATEGIES OF LB SPIROCHETES TO VECTORIAL TRANSMISSION

R₀: A MEASURE OF PATHOGEN FITNESS

At the heart of the parasite triangle (Fig. 1) is the fitness of the pathogen, quantified by the basic reproductive number, R_0 . R_0 is defined as the number of secondary infections resulting from a primary infected individual that has been introduced into a population of completely susceptible individuals [8]. If $R_0 = 1$, then the pathogen is replacing itself and is said to be endemic in the host population. If $R_0 > 1$, many secondary cases result from the primary case,

and an epizootic results. If $R_0 < 1$, then the primary case fails to infect any susceptible individuals, and the pathogen goes extinct (epidemic fadeout). Vector-borne pathogens have developed a wide range of adaptations in each of the triangle components that make them better suited to each of their tick and vertebrate host environments, which also sit in the larger external environment. Thus, to assess the contribution of adaptations to the pathogen, it is essential to examine each in the context of the overall life cycle.

R₀ EQUATION FOR THE AGENTS OF LB

Randolph and Craine [219] first developed the mathematical expression for R_0 for a tick-borne pathogen. They point out that in contrast to an expression developed for mosquito-borne pathogens, the calculation of R_0 for tick-borne pathogens must take into account the long-lived nature of ticks relative to their hosts by including tick population dynamics. The expression they developed for R_0 for LB spirochetes is:

$$R_0 = \frac{(Nf\beta_{V-T}\beta_{T-T}\beta_{T-V}p^n F)}{H(r+h)}, \tag{1}$$

where N/H is the ratio of ticks to vertebrate hosts; f is the combined probability of a tick contacting and feeding successfully on a host; β_{V-T} is the transmission coefficient for LB spirochetes from an infected vertebrate to a tick; β_{T-T} is the transstadial transmission coefficient through the molt from one life stage to the next one; β_{T-V} is the transmission coefficient for LB spirochetes from an infected tick to a vertebrate;

p is the tick’s daily survival probability; n is the extrinsic incubation period; F is the vector’s fecundity; r is the host’s daily recovery rate (i.e. the loss of the ability to transmit spirochetes to susceptible ticks); and h is the host’s daily mortality rate. From this expression, one can see that any factors that increase the values in the numerator or decrease the values in the denominator will increase R_0 and its persistence through time. In particular, because it contains an exponential term, p^n affects R_0 profoundly.

One also can see how genetic variation among strains within a population could result in different phenotypes that will experience different fitnesses, subsequently resulting in strain-specific R_0 . For each strain, R_0 will be a sum of that from each host species. Then at the population level, R_0 for a local population of LB spirochetes is equal to the sum of the individual R_0 weighted by the relative abundance of each genotype.

ADAPTATIONS AND R₀

R_0 provides a framework to assess the adaptive value of a particular phenotype of the spirochete. Any heritable trait that increases R_0 for LB spirochetes is considered an adaptation. For example, if R_0 were increased because a spirochete possesses a variation of an outer surface protein molecule that allows it to evade a vertebrate host’s immune system better than other variants, that variant would be hypothesized to be an adaptation. It is important to remember that (i) it is the *environment* that determines which variations are adaptive, and likewise, (ii) traits that are adaptive in one environment may not be as adaptive, or may even be maladaptive, in another.