Chapter from:

# LYME DISEASE AND RELAPSING FEVER SPIROCHETES

## Genomics, Molecular Biology, Host Interactions and Disease Pathogenesis

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## Chapter 10

# Perpetuation of Borreliae

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**Abstract**
With one exception (epidemic relapsing fever), borreliae are obligately maintained in nature by ticks. Although some *Borrelia* spp. may be vertically transmitted to subsequent generations of ticks, most require amplification by a vertebrate host because inheritance is not stable. Enzootic cycles of borreliae have been found globally; those receiving the most attention from researchers are those whose vectors have some degree of anthropophily and, thus, cause zoonoses such as Lyme disease or relapsing fever. To some extent, our views on the synecology of the borreliae has been dominated by an applied focus, viz., analyses that seek to understand the elements of human risk for borreliosis. But, the elements of borrelial perpetuation do not necessarily bear upon risk, nor do our concepts of risk provide the best structure for analyzing perpetuation. We identify the major global themes for the perpetuation of borreliae, and summarize local variations on those themes, focusing on key literature to outline the factors that serve as the basis for the distribution and abundance of borreliae.

**Introduction**
Whereas the term "borreliosis" comprises relapsing fever, Lyme borreliosis, and the veterinary borrelioses, great global diversity of the causative agents, as well as related spirochetes not associated with disease, has been recognized during the last three decades. In part, borrelial diversity has been recognized more easily due to profound advances in genetic characterization but also because of increased attention by researchers. Is there a similar diversity in life cycles of these borreliae? In this review, we try to describe the essential character of the borrelioses at the ecological scale, with the objective of determining whether there are a limited number of orchestral pieces or a large oeuvre of distinct pieces. Are there many different themes (Beethoven's oeuvre) or simply variations on a few themes (kazoo vs. orchestral interpretations of the 5th Symphony)? Are the local vector-pathogen-host systems unique, or are they simply local variations? In what follows, we attempt to identify the main contributors to the perpetuation of the borreliae, with the aim of analyzing the factors that serve as the basis for their distribution and abundance. This is not a comprehensive review, nor do we recapitulate other reviews on the subject, but rather emphasize specific primary literature that make points that we consider to be critical to advancing the field. The perpetuation of borreliae, of course, depends on that of their obligate vectors and vertebrate hosts, but this review is not focused on tick or animal ecology, but to the extent possible, on the spirochetes in relation to their hosts.

**Perpetuation and the zoonotic condition**
The population biology of infectious agents is centered on understanding how the basic reproduction number (BRN), in its simplest form the number of secondary infections that derive from one infection, exceeds 1 from generation to generation(May, 1984). BRN<1 implies local extinction. BRN=1 is maintenance of the agent. BRN> 1 allows for perpetuation in local space and time. The enzootic cycle refers to the dynamic biotic and abiotic associations allowing for perpetuation. (For vector borne infections and those of ticks in particular, such associations necessitate conceptualizing BRN in terms of production of new infections in the next generation, averaging between infected vectors and vertebrate hosts (Hartemink et al., 2008). We use the simple heuristic concept of BRN in this chapter). Some spirochetes of the genus *Borrelia* (herein termed borreliae) can cause infection and disease in humans (e.g., relapsing fevers and

Lyme disease) and are zoonotic, that is, they are infections maintained by animals other than humans that can infect and cause disease in humans. The term “anthroponotic” designates the maintenance of an infection that solely has humans as the critical and only vertebrate host. A simplified view of the zoonotic condition is that BRN greatly exceeds that required for perpetuation, allowing spillover or overflow from the enzootic cycle and increased probability that humans encounter infection. Of course, even in the presence of large numbers of infected ticks, there might be no human infection unless the tick vector is anthropophilic and human behavior allowed for exposure to the ticks. Not all borreliae affect human health; some are veterinary agents. Some borreliae may even be elements of a tick or vertebrate’s microbiome with no effects on the fitness of their hosts. There are also borreliae in enzootic cycles that are very unlikely to become zoonotic, or borreliae very closely related to zoonotic ones to which people are certainly exposed but evidence to date fails to confirm infectivity to humans. Other than the anthroponotic borreliae (African tick borne relapsing fever and louse borne relapsing fever), the capacity to infect humans has no bearing on perpetuation (i.e.,humans are dead end hosts for the borreliae).

*Ecology and epidemiology*

Analyses of the ecology of the borrelioses, particularly those caused by *Borrelia burgdorferi* sensu lato, have mainly had the objective of understanding human risk for acquiring infection and sustaining disease. (In practical terms, funding for ecological research is more easily justified by the risk implications.) Risk, however, has a strong human behavioral component that may skew our way of studying the natural history of the causative agents. For example, an epidemiologist would ask “what are the faunal attributes of a yard that are associated with risk to those exposed to that yard?” but an ecologist would ask “are the factors that contribute to the basic reproduction number of the agent different in a yard as opposed to the woodland?” with little interest in whether answering the question might have implications for risk. The field measurements would differ in emphasis: in the former, the entomological inoculation rate (also known as “density of infected nymphs” ) is determined by drag sampling, the fauna and its tick burden at the time of sampling is described (usually by limited cross-sectional or convenience trapping), and either or both are compared with measures of human disease incidence. Added layers of complexity might include description of pathogen genetic diversity or microbial interactions (“coinfection”). The kind of vertebrate host that is most abundant, with the greatest tick burden and greatest infectivity to ticks is identified within a site, again by limited cross-sectional or convenience trapping at specific time points and is called the “reservoir”. All too often, such a “snapshot” is considered representative of the “ecology”. Such snapshots, however, undoubtedly miss the complexity of borrelial perpetuation. With ecologists, the dynamic nature of an ecosystem is assumed: vertebrate hosts and their symbionts, as well as the animals and plants with which they interact, have varying demography within and between transmission seasons and, hence, longitudinal observations are needed. Sites, even those separated by a few hundred metres or less, may greatly differ in vegetation structure as a function of historic landscape use, local weather, geology or connectivity with other habitat patches and thus the fauna and flora may differ; such microhabitat heterogeneities comprise additional vessels in which natural selection may operate to promote perpetuation. Genetic diversity of the host, pathogen, and vector is axiomatic; natural selection operates continually.

Borreliae are obligately maintained by ticks, with one exception (louse borne relapsing fever). In some cases, they may be perpetuated by inheritance by the vector alone (in which case the vector is the reservoir) but in most cases a vertebrate amplifying host (typically called a reservoir) is required due to unstable inheritance in which infection is gradually lost with successive generations in the absence of new infection during a bloodmeal. The ecology of borreliae, therefore, comprises that of 3 distinct organisms (borrelia, arthropod, and vertebrate) and all the possible interactions between them, as well as those with other animals and plants in the environment and the physical environment itself. Even the bacterium within its tick vector might be indirectly affected by environmental stressors (e.g., prolonged exposure of an infected tick to dessication leading to its increased activity to rehydrate, thereby depleting tick energy reserves, which might lead to downregulating innate immunity or perturbation of microbiota). Borreliae would certainly be affected by the environment via the success of ticks finding and feeding on hosts, as well as anything else affecting the survival of their tick hosts, including weather or predation. Any single year’s effects may exert changes for years to come: a decrease in hosts

during the months when larvae are most active (in the example of the typical phenology of some hard ticks) within one transmission season might imply fewer resulting infected nymphs in the next year. This would lead to less spirochetal transmission (fewer infected vertebrate hosts); if there was no compensation for fewer infected vertebrates (e.g., by clustering of larvae and massive feeding on the few existing infected hosts…hence the term "amplifying hosts"), then one poor transmission year could imply poor transmission for many following years due to a chain of dependent events. In the absence of human-directed intervention, though, there has been no report of stable reduced transmission over time in any site, suggesting generally effective buffering of the transmission cycle.

There are two main phylogenetic groups of borreliae, comprising those related to the main agents of Lyme borreliosis (*Borrelia burgdorferi* sensu lato) and those causing relapsing fevers; these have classically been termed "hard tick" (ixodid) and "soft tick" (argasid) transmitted borreliae. The two groups have distinct genetic and biological characteristics(Barbour et al., 2017),and some have argued that they each merit their own genus (Gupta, 2019). Since the first studies of borreliae, relapsing fever-like spirochetes have been known to infect and be transmitted by hard ticks (the agent of bovine borreliosis), but few "hard tick" borreliae have been found in soft ticks (Lane et al., 2010) even as we have recognized additional "soft tick" spirochetes within "hard ticks". There is now evidence fora third phylogenetic group that does not cluster within the two classical clades, that of borreliae infecting reptiles and specific reptile feeding ticks or monotremes and their ticks(Loh et al., 2016; Takano et al., 2010), which are generally hard ticks.

The general paradigm of borrelial life cycles is that Lyme disease spirochetes are maintained by rodents, eulipotyphlans (formerly "insectivores" including shrews and hedgehogs), birds and *Ixodes* ticks and that relapsing fever spirochetes are maintained by nest or burrow inhabiting mammals (rodents or eulipotyphlans) and argasid (mainly *Ornithodoros*) ticks. There are major differences in these life cycles: with the former, vertebrates are generally infectious for extended durations; *Ixodes* ticks take several days to take a large bloodmeal, drop off, develop over a long time, often several months, to the next bloodfeeding stage with periods of dormancy. Many different kinds of vertebrates might be fed upon. Adult stages of *Ixodes* ticks may feed on larger hosts that are less susceptible to spirochetal infection, if at all.

With relapsing fever spirochetes, infectivity is thought to be of limited duration due to eventual immune control even with antigenic variation; soft ticks, with a few exceptions, take a few hours at most to feed and may become ready to feed again within a few weeks, with there being as many as 5 nymphal stages (and even multiple small bloodmeals within a nymphal stage) and multiple smaller bloodmeals by the female ticks resulting in many small egg batches. Soft ticks may feed on the same kind of animal, even the same individual, at any stage of their life, and their host range is restricted by whatever inhabits or visits infested burrows or sleeps near the ticks' hiding places. However, unlike hard ticks, soft ticks may survive for years in the absence of a bloodmeal, as does their capacity to transmit borreliae. Both the multihost (typically known as "three host") tick life strategy and the nidicolous (nest-inhabiting) "one host"strategy have obviously allowed for the extensive adaptive radiation and perpetuation of the borreliae.

**_Borrelia burgdorferi_ sensu lato: are there as many modes of perpetuation as there are genospecies or just variations on a theme?**

The *Borrelia* entry in the NCBI taxonomy server https://tinyurl.com/y3bb7q7f lists 23 species of *B. burgdorferi* s.l. ("Borreliella") with additional *Candidatus* and as yet undescribed cognate sequences. We do not examine each of these but analyze those that provide examples of concepts that are the basis for understanding the perpetuation of these spirochetes.

*Is there a general enzootic paradigm?*

The universal association is that of *B. burgdorferi* s.l. with a tick in the genus *Ixodes*. Although *B. burgdorferi* s.l. has been reported to infect other genera of ticks, subsequent molecular analyses of the agent that was detected or experimental transmission experiments do not support the role of other tick vectors. Metastriate ticks such as *Dermacentor variabilis* do not support *B. burgdorferi* due to upregulation of hemolymph antimicrobial peptides and increased phagocytic activity (Johns et al., 2001). Indeed, the obligate binding of the major *B. burgdorferi* outer surface protein (OspA) with the *Ixodes* gut cell receptor TROSPA, promoting spirochetal infection of the vector(Pal et al., 2004), suggests that there is a very specific association of

*B. burgdorferi* with *Ixodes* ticks. It is noted, however, that TROSPA homologs occur in other tick genera (Urbanowicz et al., 2016) and thus their role as possible vectors cannot be completely dismissed.

*Transovarial transmission (TOT)*

Some vector-borne agents may be inherited by progeny from an infected female, a process known as transovarial or vertical transmission. Early studies of host seeking *I. scapularis* and *I.ricinus* larvae detected spirochetes by immunofluorescence and, in the absence of evidence that larvae might briefly attach to a host and become infected, then detach and seek hosts again, suggested that *B. burgdorferi* s.l. could be inherited (Piesman et al., 1986; Rijpkema et al., 1994). The comprehensive report demonstrating that *B. miyamotoi* was sympatric in most sites with *B. burgdorferi* s.l. and that it was maintained by TOT (Scoles et al., 2001) led to a reexamination of transovarial transmission. Over 100 pools of questing *I.ricinus* larvae yielded none with evidence of *B. burgdorferi*s.l. from Czech and German study sites (Richter et al., 2012)finding only *B. miyamotoi.* Rollend et al. (Rollend et al., 2013) analyzed 1214 pools of larval ticks (50 larvae per pool, each pool representing a single fed female ticks collected from field sites) by PCR and did not find any evidence of *B. burgdorferi* s.s. infection, but 1.4% contained evidence of *B. miyamotoi.* However, Van Duijvendijk et al. (van Duijvendijk et al., 2016) demonstrated that host seeking *I. ricinus* were infected by *B. afzelii* (0.62% of 1456 larvae) and that the larvae were infectious to mice, with *B. afzelii* recovered from skin of the mice. In their study sites, based on estimated larval and nymphal burdens on mice, it appears that larvae could account for as much as a quarter of all rodent infections due to the sheer number that feed on these hosts, assuming the same 0.62% prevalence of infection. TOT, thus, could contribute to BRN and might facilitate the establishment of new enzootic sites when infected ticks are introduced.

*Is there a general vector?*

What is clear is that for zoonotic transmission, the most important vectors are ticks in the *Ixodes persulcatus*/*ricinus* species complex, which comprises the species *I. persulcatus*, *I. ricinus*, *I. scapularis*, and *I. pacificus*, among others (Charrier et al., 2019). These ticks tend to share a general life cycle with adults feeding on larger animals, mainly deer, and the subadults on a wide range of small mammals, birds, and even reptiles. The full life cycle may be completed in 2 years, although those of *I. persulcatus* may extend for 4-6 years, depending on climatic zone (Shashina, 1985). There is marked seasonality for the activity of each life stage. Adult ticks are most common in colder months, from fall to spring. Larvae seek hosts in the summer, nymphs in the spring and early summer. There are species specific variations to this general temporal sequence reflecting the wide range of sites and habitats for this group of ticks. Temperature and photoperiod drive the life cycle via effects on development and host seeking behavior. These extended developmental or behavioral delays (diapauses, Greek “diapausis, = pause) between stages are a mechanism that allows adaptation to difficult environmental conditions. In the laboratory, all of these ticks may be induced to develop from egg to adult within 7 months at temperatures much greater than they would experience in nature (Ogden et al., 2004). At 21ºC, a typical “room temperature” at which many tick biologists keep their colonies, *I.scapularis* eggs are deposited by the replete female and eclose (hatch) within 3 months; they may feed within the month after eclosion; the engorged larvae molt to nymphs in a month; the nymph may feed within the month after molting; and the engorged nymph can develop into adults within 2 months.

The critical attribute of a zoonotic vector is anthropophily. Because *B. burgdorferi* undergoes reactivation (Schwan and Piesman, 2000) and, hence, the probability of transmission increases with duration of attachment (Piesman et al., 1987), the prompt removal of an attached tick modifies apparent risk, implying that larger size ticks (adults) are less effective vectors than are smaller ones (nymphs). Interestingly, there is variation among the species complex as to the stage during which anthropophily is greatest, which influences zoonotic risk. In Russia, the main vectors are adult female *I. persulcatus* because the nymphal stage rarely attaches to humans (Korenberg, 1994).

Although the vector in the eastern U.S.is universally considered to be *I. scapularis*, there are at least 2 genetically distinct lineages or mitochondrial haplogroups (Qiu et al., 2002; Rich et al., 1995; Sakamoto et al., 2014; Xu et al., 2020), and likely as many as 5 (Gulia-Nuss et al., 2016). We remain the only research group continuing to refer to the “northern” lineage as *I. dammini* (considered the junior subjective synonym of *I. scapularis*) to remind workers that the more southerly populations of *I.*

**Figure 1.** Nymphal *Ixodes dammini* seeking an attachment site, human skin.

*scapularis* rarely bite humans in the nymphal stage (Goddard and Piesman, 2006; Merten and Durden, 2000). Lyme disease risk in central and southern states is notably small even with known enzootic transmission (Gulia-Nuss et al., 2016). By contrast, infection by nymphal *I. dammini* (Figure 1) is the hallmark of the epidemiology of Lyme disease in New England and Upper Midwest sites of intense zoonotic transmission. Whether this behavioral difference (anthropophily), which is the basis for zoonotic transmission of *B. burgdorferi*, is genetically based remains to be explored (Telford III, 1998), although some SNP-defined clades of ticks are not represented in Lyme disease endemic states (Gulia-Nuss et al., 2016). During the last 30 years, "*I.scapularis*" has greatly expanded its range in part due to bird transport (Eisen et al., 2016), and both major lineages are now present in many central and southern U.S. sites (Sakamoto et al., 2014). A critical test of whether there is merit in distinguishing between the lineages is to determine whether human infestation increases where *I.scapularis* was previously known to only rarely bite humans; we predict that those ticks removed from people, if genetically characterized, will comprise the apparently more anthropophilic invasive northern lineages.

Of course, these considerations are not relevant to the perpetuation of *B. burgdorferi* unless anthropophily covaries with host choice or vector competence. The two lineages do not differ in vector competence (Dolan et al., 1997; Jacobs et al., 2003). Although *I.scapularis* appears to mainly feed on lizards in more southerly sites, it is not clear whether this is a specific choice or opportunism inasmuch as the diversity and abundance of lizards is significantly greater in more southerly sites than in middle Atlantic or northeastern American sites. *I. dammini* from Massachusetts feeds well on different kinds of lizards in the laboratory (unpublished). Interestingly, in experimental outdoor microcosms, nymphal ticks from northern populations were 8 times more likely to seek hosts above the leaf litter than those from southern populations, suggesting an adaptation of the latter to infesting *Scincella lateralis* lizards (Rogers, 1953)that dwell within leaf litter in those southern sites (Arsnoe et al., 2019). Warmer temperatures in more southerly sites, as well as less dense small mammal populations, may have influenced host seeking behavior of *I.scapularis* (Ginsberg et al., 2017), thereby helping to explain the generally lower force of *B. burgdorferi* transmission there (Bowman et al., 2009; Little et al., 2010; Xu et al., 2020). American lizard species are thought to be poorly competent for *B. burgdorferi* infection (Lane and Loye, 1989; Lane and Quistad, 1998), and this suggestion has been used to help explain the relatively low enzootic and zoonotic transmission in southerly sites where lizards are common. However, there are diverse reports that provide exceptions to the "lizards are incompetent reservoirs" dogma (Clark et al., 2005; Levin et al., 1996; Swei et al., 2011), and it may be that lizards serving as major hosts for *I.scapularis* subadults has only a minor or indirect role in transmission of *B. burgdorferi* in more southerly sites. The Eurasian *B. lusitaniae* is maintained by lacertid lizards and *I. ricinus* (Richter and Matuschka, 2006), demonstrating that lizards are not generally incompetent for *B. burgdorferi* s.l.

*Early paradigms (the Northeastern U.S. model) for* B. burgdorferi *.s.s.*

By the time an association of Lyme arthritis with a specific tick had been determined (Wallis et al., 1978), studies of the epidemiology and ecology of human babesiosis due to *Babesia microti* (Nantucket Fever) were well underway on Nantucket Island, Massachusetts by Andrew Spielman and Joseph Piesman. They incriminated *I. dammini* as the vector, and the white footed mouse, *Peromyscus leucopus* (Figure 2), as the main reservoir of the protozoal agent. Piesman and Spielman (Piesman and Spielman, 1979) identified the main host associations and basic phenology (larvae in late summer, nymphs

**Figure 2.** White footed mouse, *Peromyscus leucopus*, infested by larval deer ticks (ear).

in late spring, adults from fall to spring) and by the time *I.dammini* was also incriminated as the vector of Lyme arthritis, the major features of the tick's life cycle and the ecology of *B. microti* had been determined. It was not long thereafter that the suggestion was made that the main elements of the ecology of both infections were essentially the same (Spielman et al., 1984). Subsequent reports out of the Harvard lab on the reservoir capacity of this mouse (Donahue et al., 1987; Levine et al., 1985) for the "*Ixodes dammini* spirochete", soon to be named *B. burgdorferi*, established the paradigm of a mouse-driven zoonosis. The prevalence of *P. leucopus* on Nantucket Island, where there were fewer kinds of small mammals compared to mainland sites, and the strength of the evidence of its role in the ecology of *B. microti* there, biased early investigators as to how they thought of the perpetuation of *B. burgdorferi* in northeastern North America. White footed mice were considered to be the main reservoir.

Spirochetal isolates were obtained from most kinds of small mammals endemic to mainland southern New England, as well as from rabbits, mustelids, carnivores, and passerine birds (Anderson, 1988). The Harvard group tended to dismiss such findings as merely evidence of infection but not of reservoir capacity, insisting on the gold standard of xenodiagnosis (the feeding of uninfected laboratory-reared larval deer ticks on reservoir candidates) to confirm a reservoir. Subsequent work demonstrated the reservoir competence of shrews and rabbits (Telford III and Spielman, 1989; Telford et al., 1990), certain passerine birds, but not others (Mather et al., 1989a), and even voles and chipmunks (Mather et al., 1989b). Nevertheless, the Harvard group continued to insist that *P. leucopus* was the main reservoir and that others contributed considerably less to the overall population of infected ticks. Other hosts fed fewer subadult deer ticks (average larval burdens of >10 for mice vs. <2 for chipmunks or shrews) and were less infectious (typically >70% of xenodiagnostic ticks feeding on mice became infected but <50% of those feeding on shrews or chipmunks). Mice were also as much as 10 times more abundant than any of these other species in sites where deer ticks were common. In addition, mice were immunotolerant to tick infestation whereas other hosts such as voles or chipmunks tended to reject subadult *I. dammini* after an initial infestation (Davidar et al., 1989; Mather et al., 1989b), hence would fail to feed sufficient numbers of ticks to contribute to BRN. It should be noted, though, that the snapshot nature of such analyses fail to examine the full range of age of potential hosts; voles or chipmunks may be more tolerant of infestation or more infectious as recruits. Then too, immuno-tolerance to tick infestation has been measured with large numbers of ticks being fed on a host, as opposed to the likely "trickle" infestations (a few ticks every few days) that the majority of wild animals sustain. Rodents in general have rapid demographic turnover, so the extended duration of reservoir capacity for mice may not necessarily be as influential as expected.

On the other hand, deer had been determined by experimental infection attempts to be insusceptible to infection by *B.microti* (Piesman et al., 1979) and their reservoir incompetence for *B. burgdorferi* was demonstrated by xenodiagnosis (collecting replete larvae that naturally infested deer in intensely enzootic sites and allowing them to molt to nymphs, which were assayed for infection (Telford III et al., 1988). Such quantitative arguments were generated, in part, by trying to understand the conditions required for the excess BRN that leads to zoonotic overflow.

The central role for *P. leucopus* in the enzootic cycle of *B. burgdorferi* in the northeastern U.S. has its basis in a theoretical argument. Because ixodid ticks feed but once in each developmental stage, in the absence of inherited infection, a pathogen's transmission would depend on a vector focusing its feeding on a single kind of competent host (larvae that becomes infected must transmit as a nymph to a competent reservoir, otherwise the infection does not contribute to BRN). Narrowness of mosquito host

range is the most influential of the variables in the malarial vectorial capacity model (Spielman and Rossignol, 1984). This seminal model greatly influenced Spielman, who used considerations of narrowness of host range to argue for a single most important reservoir for *B. microti* and *B. burgdorferi* (Spielman et al., 1984; Spielman, 1988). Such considerations directly led to the concept of a zooprophylactic (Russell, 1934) or dilution effect, that community host diversity will dilute the effect of white footed mice in producing infected ticks (Spielman et al., 1984; Spielman, 1988; Ostfeld and Keesing, 2000) and thereby human risk. Although data from insular sites (particularly Nantucket Island) tended to support this concept (Spielman et al., 1984), narrowness of vector host range does not seem to characterize the perpetuation of *B. burgdorferi* s.l. whose global distribution and abundance may be related more to adaptive plasticity: the spirochete and the tick are opportunistic. Indeed, the role of other species such as rabbits, shrews or chipmunks in buffering the perpetuation of eastern North American *B. burgdorferi* during times when or where mice might be scarce has been amply documented (Telford III and Spielman, 1989; Telford et al., 1990; LoGiudice et al., 2003; Brisson et al.; 2008).

Then, too, an adaptationist perspective is common among biologists, with natural selection caricatured as producing the most optimal solution (Gould and Lewontin, 1979). Enzootic and zoonotic transmission of *B. burgdorferi* should be optimal only with a tick that focuses its subadult feeding on a highly reservoir competent host. Such a host would allow the production of a great number of infected ticks, which made it more likely that BRN>>1 and the more likely that humans might be exposed to infection. But, is perpetuation really compromised by suboptimality? One might argue that if infection rates were small for host-seeking nymphs, the probability of encountering a susceptible individual mouse would be correspondingly diminished relative to when more infected nymphs were available. The 20-80 rule of parasitology, an empirical and theoretically based principle that posits 20% of the hosts are infested or infected by 80% of the individual parasites (Woolhouse et al., 1997) applies to ticks (Randolph et al., 1999) and undoubtedly helps maintenance of *B. burgdorferi*. Small numbers of mice in our Nantucket field sites feed most of the *I.dammini* larvae and nymphs (Figure 3). But, the basis for why an individual animal becomes heavily infested and the majority do not has not been explored.

*Complexity is increasingly recognized*: *California*
Evidence of complexity and of local differences in perpetuation of *B. burgdorferi* was provided in other regions nearly concurrently with the early New England studies. The western blacklegged tick, *Ixodes pacificus*, is the main vector for *B. burgdorferi* in the western USA and British Columbia (Lane et al., 1991). Lyme disease is more focal in its distribution in these areas, with most cases reported from the northern coastal counties of California. Seminal studies by Robert Lane and colleagues identified *I. pacificus* and *I. spinipalpis* (at the time, *I. neotomae* was implicated (Brown and Lane, 1992), but the infesting ticks were subsequently identified as *I. spinipalpis*) and relatively long lived rodents such woodrats and kangaroo rats, but only rarely *Peromyscus* spp. The study site was mainly the University of California Hopland Field Station in Mendocino County, which comprises chaparral habitat. *I. spinipalpis* only rarely bites humans, but *I. pacificus* was thought to serve as the zoonotic bridge by feeding on infected woodrats and kangaroo rats as well as humans. At the time, this paradigm served as a stark contrast to the *P. leucopus* driven transmission by an aggressive *I. dammini* vector and served as evidence that *B. burgdorferi* enzootic cycles vary geographically. Epidemiological studies determined that California Lyme disease cases were exposed mainly in dense woodlands (Clover and Lane, 1995) where woodrats and *Peromyscus* were infrequently infected by *B. burgdorferi* (Wright et al., 2000).

California is the most biodiverse state in the U.S., with 7 different biomes comprising 21 distinct ecological communities (Schoenherr, 1992). An analysis of mtDNA identified great haplotype diversity in California populations of *I. pacificus*, with many mtDNA haplotypes unique to sampling sites (Kain et al., 1999). In this context of small local lineages of vector ticks within diverse ecological communities, the finding that *B. burgdorferi* comprises a species complex in California should not be surprising. *B. burgdorferi* s.s., *B. bissettiae*, *B. americana*, *B. lanei*, *B. californiensis*, and other as yet unnamed genospecies have been isolated from *I. pacificus* and *I. spinipalpis* (Fedorova et al., 2014; Postic et al., 2007), but patterns of association between borreliael genospecies, vertebrate hosts, and tick lineages remain to be described. Most Lyme disease in California is caused by *B. burgdorferi* s.s., although there are case reports that suggest *B. bissettiae* may be an etiologic agent. Woodrats in chaparral and

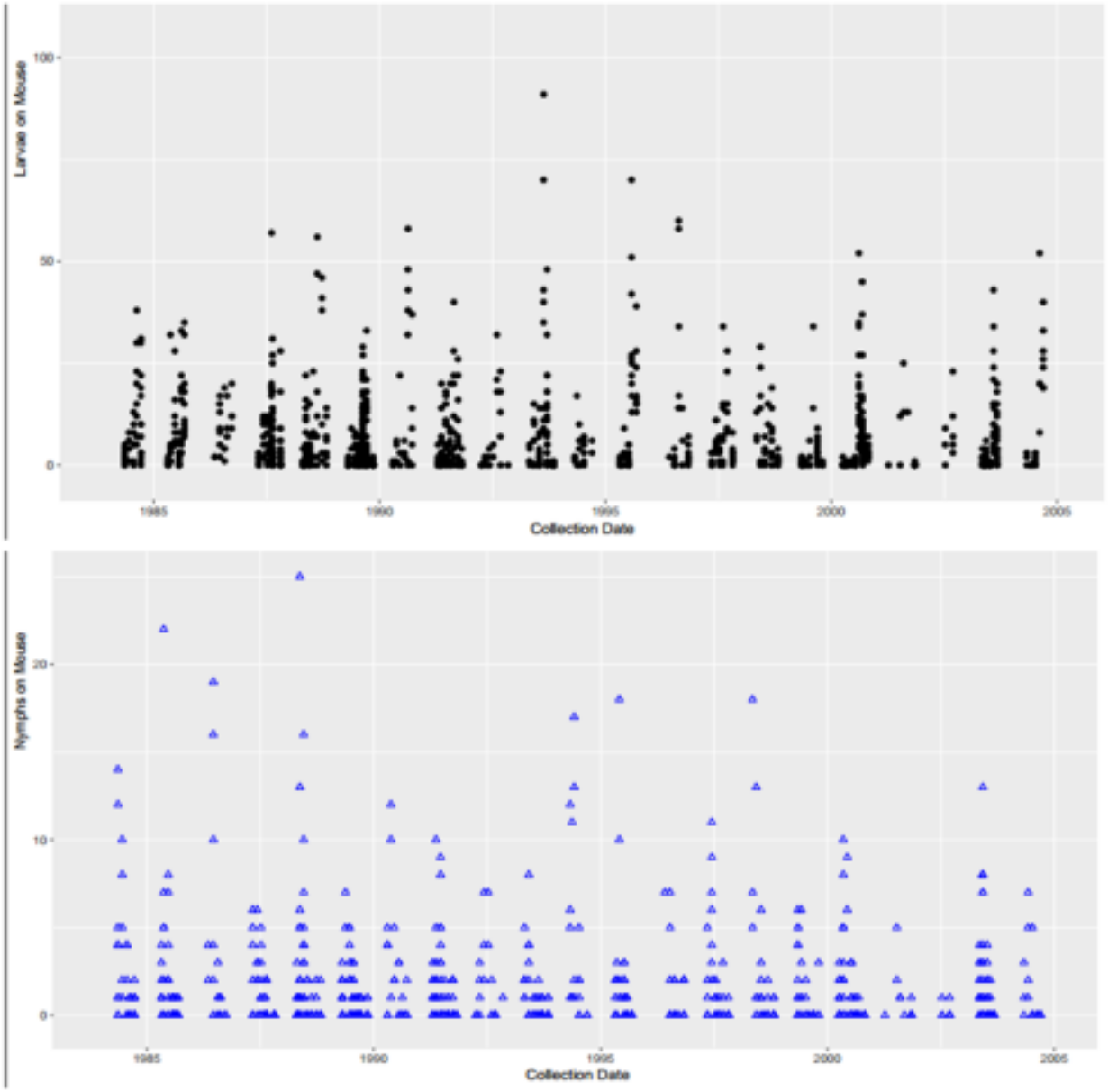


**Figure 3.** 20:80 rule. Infestation indices on *P. leucopus,* Nantucket Island, 1985-2004. Mice were trapped monthly (AMJJAS) on one acre grids. X axis, time. Y axis, number of ticks. Each dot represents the tick burden of a single mouse. Top graph is *I.dammini* larvae, bottom nymphs.

grassland sites maintain *B. bissettiae* or other *B. burgdorferi* s.l. (Postic et al., 1998, 2007; Vredevoe et al., 2004). Kangaroo rats maintain *B. californiensis* likely with *I. jellisonii* as the vector (Lane and Brown, 1991; Postic et al., 2007) although *I. spinipalpis* and *I. pacificus* also feed on this heteromyid rodent in the chaparral habitats of California. Of particular interest is the fact that western gray squirrels (*Sciurus griseus)* maintain *B. burgdorferi* s.s. in oak woodlands (Lane et al., 2005), suggesting that human risk might be promoted by the known tendency of this species to inhabit periurban and suburban habitats.

*I. pacificus* is found mainly in the summer Mediterranean climate biomes in California, but ranges into Washington, British Columbia and western Canada, with isolated populations in Arizona,

Utah, and Nevada. In California, the life cycle is extended, with adults seeking hosts in the fall and winter, fed females ovipositing in late winter through spring, and eggs eclosing in mid-late summer. The larvae do not immediately seek hosts, but overwinter and become active in the early spring, with a peak in May and April. Engorged larvae molt immediately, but the nymphs are inactive until late winter and feed through the spring. Fed nymphs will molt to adults in the late summer. Hence, the life cycle is a minimum of 3 years (Padgett and Lane, 2001).

*Eurasian Lyme disease spirochetes*

The main vector of Lyme disease spirochetes in Europe is the castor bean (sheep) tick, *I. ricinus*. This widely distributed tick, ranging from Ireland in the west to Scandinavia and the Russian Federation to St. Petersburg in the east, to the Mediterranean including parts of western North Africa, can be found in diverse habitats, even pasture, although it prefers heavily forested sites that provide the humid microhabitat required for development and survival. *I. ricinus* is recorded as infesting 44 different species of mammals, birds, and reptiles, but the majority of these ticks in continental Europe feed on roe deer, small rodents, and blackbirds (thrush family) (Hofmeester et al., 2016). In the southern part of its range, *I. ricinus*, like *I. scapularis*, may heavily infest lizards (Amore et al., 2007).

The life cycle of *I. ricinus* varies across its range. In the British Isles, where classic studies were undertaken of its natural history (Gray, 1984; Lees and Milne, 1951; Milne, 1943),there is a bimodal activity period for each of the stages, corresponding to spring and fall feeding cohorts. Adults seek hosts once temperatures reach 7ºC in the spring, with peak activity in May and June. A smaller peak is observed in August and September. Eggs from the spring cohort eclose in the summer, but this may be delayed until the following spring as it is for those from females feeding in the fall. Larvae feed in May and June, with a smaller peak in August and September. Nymphs generally follow the same activity pattern. Engorged larvae and nymphs will molt in the summer, but diapause until the following spring. Throughout its range, there is large overlap in seasonal activity between the stages, but such overlap may vary with latitude.

The identification of *B. burgdorferi* as the etiologic agent of Lyme disease included *I. ricinus* collected from Switzerland (Burgdorfer et al., 1982), and comprehensive studies there found infection in 5%-34% of host seeking ticks. In addition, *Apodemus* spp. mice were found to be infectious to laboratory reared ticks (xenodiagnosis), incriminating them as reservoirs (Aeschlimann et al., 1987). Strain heterogeneity was quickly recognized by European workers, distinguishing patient isolates from Europe from those of the U.S. by monoclonal antibody typing and by ultrastructure (Hovind-Hougen, 1984; Wilske et al., 1988). We now know that in Eurasia, *B. burgdorferi* s.l. includes over 20 genospecies, but only 3 cause the vast majority of human disease (*B. burgdorferi* s.s., *B. afzelii*, and *B. garinii*).

Population genetics analyses demonstrated little population structure for *I.ricinus* across northern and central Europe (Meeûs et al., 2002). Some isolated populations may be distinguishable (e.g., Great Britain from Latvia and Western Norway (Dinnis et al., 2014; Røed et al., 2016). Although the English Channel is a physical barrier to tick movement, birds may easily transport ticks great distances and, thus, the low degree of apparent divergence was not expected. This finding has been attributed to the very small likelihood that introduced subadults from birds would generally fail to reproduce; one estimate for developmental success between stages is <10% (*I*.e., 90% of all ticks at each stage die (Randolph et al., 2002). One exception to the rather homogenous population structure across continental Europe is that *I. ricinus* from southwest Mediterranean and North African sites appeared to be partially genetically isolated. These analyses led to re-examination of the Mediterranean "*I. ricinus*" and the recognition of a new cryptic species, *I. inopinatus* (Estrada-Peña et al., 2014). *I. inopinatus* has been found in northern and southern Germany and, in fact, is as frequently infected as *I. ricinus*, long known to be the only vector there (Hauck et al. 2019).

The diverse genospecies in Europe may be grouped into 3 categories with respect to their reservoir host: generalist, rodent specialized, and bird specialized. These categories derive from data on isolation (or DNA evidence) from or xenodiagostic studies of wild-caught animals, associations of animal species with particular sites, prevalence of infection in host-seeking ticks, and experimental infection. Of note is that only *B. burgdorferi* s.s. is considered a generalist, infecting rodents as well as birds. *B. burgdorferi* s.s. is the dominant genospecies in North America, but is less frequently encountered in Europe (relative to *B. afzelii* and *B. garinii*; (Strnad et

al., 2017) for as yet undescribed reasons. *B. burgdorferi* s.s. is more frequently detected in adult *I. ricinus* than in nymphs (Mysterud et al., 2019), suggesting that this spirochete is likely associated with a host such as squirrels that feeds more nymphs than larvae (Humair and Gern, 1998).

In much of Eurasia, the vector for *B. burgdorferi* s.l. is *I. persulcatus*. The taiga tick ranges from the Baltic Sea to Japan, with the center of infestation within the Russian Federation, although half the provinces of China have known infestations (Chen et al., 2010). Infestations in Japan are most common in Hokkaido, although it exists in montane sites of Honshu and Kyushu (Takada et al., 2001). As the name suggests, the tick is most common in the taiga (boreal coniferous forests, typically with pine, larch and spruce) biome, but in the western part of the range deciduous forests are infested. The duration of the life cycle depends on temperature as well as photoperiod, adaptations that are not unexpected given its large latitudinal range (from roughly 35 to 65 N latitude, although the more southerly sites are in mountains). All stages seek hosts from spring through summer, with the peak during late May and mid-June. Subadults that engorge during May and June will molt during the summer but not seek hosts, overwintering to the next year. Subadults engorging during June and July will overwinter before molting in the spring. For all stages, a decreasing photoperiod will trigger diapause, either developmental or behavioral (Babenko, 1985; Belozerov, V.N., 1985).

The host range, as for all the *I. persulcatus* complex ticks, includes a wide range of mammals (>100 species), birds (175 species), and even some lizards (Labzin, 1985; Naumov, 1985). Larvae tend to feed on mice, voles and shrews; nymphs on chipmunks, hedgehogs, and birds; and adults on larger mammals such as hare or deer, although cattle also help maintain *I. persulcatus* infestations. During times when typical larval hosts are scarce, they may densely infest hares (Uspensky and Rubina, 1992). Hence, *I. persulcatus* is an opportunistic feeder.

Analyses of *I. persulcatus* population structure reveal low diversity, 1.14% within 12S rDNA, across its range from the west to Far East, including central Russia (Kovalev and Mukhacheva, 2012). As with *I. ricinus*, this may be explained by severe bottlenecking of genetic diversity during glaciation with rapid expansion of the populations during the 10,000 years after glacial retreat. The rapid expansion was certainly facilitated by intensive agriculture and the movement of large domestic animals. *I. persulcatus* appears to be increasing its range, first appearing in Finland in the early 2000s and then in the Norbotten archipelago (northern Sweden) 10 years later (Jaenson et al., 2016). This is the most northern infestation known for *I.persulcatus* and is interpreted by some as evidence for the effects of increasingly warmer temperatures in northern latitudes.

*I. pavlovskyi*, a tick that is parapatric and sympatric with *I. persulcatus* in many sites across Russia, was found to be infected by *B. afzelii* at a prevalence greater than that of the latter in Tomsk (Korenberg et al., 2010). Subsequent analysis of the ticks around Tomsk demonstrated that 10% of 783 ticks represented natural hybrids of *I. pavlovskyi* and *I. persulcatus*. These ticks apparently form fertile F1 and may be backcrossed (Kovalev et al., 2015), suggesting that it is simplistic to consider formation of fertile F1 hybrids between *Ixodes* ticks evidence for conspecificity. Additional research is required to determine whether hybrid *Ixodes* spp. have any implications for enzootic or zoonotic transmission of *B. burgdorferi* s.l. Subadult *I. persulcatus* and *I. pavlovskyi* feed on small mammals, often the same individual host, but the adults feed on different animals, viz., ungulates for *I. persulcatus* and birds for *I. pavlovskyi* (Korenberg et al., 2010), which suggests a means of coexistence via niche partitioning.

*A* Borrelia *for every kind of* Ixodes*?*

There are 235 species of ticks comprising at least 15 subgenera within the genus *Ixodes* (Camicas et al., 1998). Eight of these subgenera have been implicated as enzootic vectors for at least one *B. burgdorferi* s.l. (*Ceratixodes*, *Eschatocephalus*, *Pholeioixodes*, *Amerixodes*, *Exopalpiger*, *Ixodes*, *Trichotoixodes*, and *Partipalpiger*). To our knowledge, borreliae have not been reported from the particularly diverse *Scaphixodes* and *Afrixodes* (31 and 60 species, respectively), nor from the less speciose subgenera. The natural history of the vast majority of *Ixodes* spp. has not been completely described, although many are very host-specific (or at least circumstantially host specific) or nidicolous, thereby cryptically maintaining enzootic cycles. Although the subgenus *Ixodes* (which includes the *I.ricinus* species complex) has been the focus of much attention, enzootic *B. burgdorferi* s.l. cycles have been recognized for relatively host specific ticks such

as the pelagic bird infesting *I. uriae* or the hedgehog tick *I. hexagonus* (Gern et al., 1997; Olsen et al., 1995). The nidicolous *I. trianguliceps* was found to be infected by *B. burgdorferi* s.l. 5-10 times less frequently than co-infesting *I. persulcatus* (the study was performed by analyzing ticks removed from trapped rodents), suggesting that it is less competent in pathogen uptake and/or spirochetal gut colonization (Korenberg et al., 2015). In Europe, *I. canisuga, I. frontalis*, *I. arboricola,* and *I. ventalloi* have all been found to be infected by *B. burgdorferi* s.l. but their role in maintenance is not clear. *I. ventalloi* is widely distributed in the southwestern Mediterranean, and feeds on diverse animals even though it is considered a “rabbit tick” (Santos and Santos-Silva, 2018; Tomassone et al., 2013). This tick is frequently found on cats, which implies that it could be common in peri-domestic sites and, thus, serve as a zoonotic bridge. However, even though *I. frontalis* and *I. arboricola*, which are bird-infesting species, are competent to acquire infection from birds and *B. burgdorferi* is transstadially maintained, they failed to actually transmit (Heylen et al., 2014). Thus, finding infection in a tick, even demonstration of positive xenodiagnosis, does not necessarily suggest it has a role in maintaining *B. burgdorferi* s.l. Nonetheless, it is almost a certainty that additional new *B. burgdorferi* s.l. enzootic cycles will be identified as the natural histories of the 200 or more valid understudied *Ixodes* species are examined.

*Factors influencing host relationships*

One might argue whether ticks are the reservoir (infection is maintained for long durations, given the extended tick life cycle) or the vertebrate is the reservoir because it can amplify infection (a single infected animal could give rise to dozens or hundreds of infected ticks) and thereby influence the BRN. Early in the studies of Lyme disease ecology, vertebrate hosts were classified as incompetent or competent reservoirs (Donahue et al., 1987; Telford III et al., 1988), usually based on whether xenodiagnostic ticks became infected either on experimentally infected or naturally exposed animals. Reservoir capacity comprised those with high reservoir competence (if a host is infected, most ticks feeding on that host become infected; and, the host does not become immune). They were densely infested by the relevant tick stage and did not become sensitized to the bites (immune rejection of the ticks). Theoretical considerations suggested that there must be “main” and ancillary reservoir hosts. The increasing recognition of borrelial genetic diversity has also been so influenced, with adaptation of genospecies to specific hosts by means of evading species-specific complement lysis. These paradigms need to be reexamined.

*Cofeeding transmission*

The BRN of *B. burgdorferi* s.l. depends on horizontal or transstadial transmission (Hartemink et al., 2008), i.e., a larva becomes infected by feeding on an infectious host that is systemically infected; spirochetes are maintained through the molt to the nymphal stage and are transmitted to a new host by nymphal feeding. However, nonsystemic or cofeeding transmission may play a secondary role in perpetuation. In this mode of transmission, uninfected ticks feeding next to infected ones may become infected without the agent needing to establish a persistent infection resulting in a bloodstream or skin infection (Jones et al., 1987). Ticks are highly clustered on hosts (Randolph et al., 1999). Cofeeding is the main mechanism for the perpetuation of tick borne encephalitis virus(Labuda et al., 1993) which only produces a transient viremia in the vertebrate host. The great overlap in seasonal activity of *I. ricinus* drives the force of TBEV transmission (Randolph et al., 1999), with infected nymphs serving as a source for larvae feeding on the same host. Cofeeding is thought to be of lesser importance for *B. burgdorferi* s.l. which generally produces a chronic infection in the vertebrate host. Sheep, which do not become systemically infected by *B. burgdorferi*, nonetheless may infect ticks via cofeeding (Ogden et al., 1997). Our recent host bloodmeal analyses suggest that some ticks may indeed become infected from deer (Goethert et al. submitted) which have long been thought to be incompetent hosts. The significance of cofeeding also includes the maintenance of *B. burgdorferi* diversity within a site where synchronous feeding of *I.dammini* larvae and nymphs is common(States et al., 2017)by promoting the survival of strains that are more rapidly cleared from the host.

*Complement sensitivity or evasion as the main determinant for* B. burgdorferi *s.l. host associations.*

A pervasive paradigm in the ecology of *B. burgdorferi* s.l. is that the adaptive radiation of these spirochetes into the great diversity of genospecies that we recognize today was driven and is maintained by adaptation or lack thereof to attack by host complement. In a seminal paper, Kurtenbach and colleagues (Kurtenbach et al., 1998) demonstrated specific borreliacidal activity when serum of diverse

animals was incubated with *B. burgdorferi* s.l. strains. This borreliacidal activity was eliminated by heating serum, or supplementation of the serum with EDTA, suggesting the role of the alternative complement pathway. Mouse serum lysed *B. garinii*, bird serum lysed *B. afzelii*, and deer serum lysed all genospecies that were tested. The pattern of genospecies specific lysis is consistent with the prevailing views of reservoir competence, e.g., that deer were incompetent, that mice were never infected by *B.garinii*, and that birds were not infected by *B. afzelii.* Cattle, whose serum lyses all genospecies, are zooprophylactic (Gassner et al., 2008; Richter and Matuschka, 2010).

Spirochetes may evade complement lysis by binding the complement regulatory protein factor H (fH) (Kurtenbach et al., 1998). Factor H is a cofactor for factor I-mediated cleavage of C3b and bound Factor H will promote the degradation of C3b and thus inhibits the remainder of the complement cascade and formation of the membrane attack complex (MAC). *B. burgdorferi* s.l. are now known to have an array of outer surface proteins that target different modulators of the 3 different complement pathways (Kraiczy et al., 2004). The *B. burgdorferi* s.l. proteins involved in modulating the alternative complement pathway are called FH/FHL-1-binding complement acquiring surface proteins (CRASPs), and comprise CspA, CspZ, and OspE-related proteins (members of the Erp family) (Kraiczy et al., 2004). BBK32 and OspC also bind elements of the classical and lectin complement pathways and inhibit the formation of MAC (Garcia et al. 2016; Caine et al. 2017). CRASPS, OspC, and BBK32, among other *B. burgdorferi* s.l. proteins, may provide functional redundancy that would allow for some degree of plasticity in host associations and may be the basis for the leakiness of the complement lysis theory for host adaptation.

There are natural exceptions to the expected host associations: grey squirrels, for example, may be infected by *B. garinii* (Millins et al., 2015), and some inconsistencies from predicted host ranges for genospecies were detected when host seeking nymphal *I. ricinus* were analyzed by host bloodmeal analysis (Cadenas et al., 2007). The theory of differential sensitivity to host complement as a driver for the known genospecies/host associations should be viewed in the context of the fact that analyses of complement lysis (e.g., (Bhide et al., 2005) do not demonstrate 100% lysis but rather report the data as >90% lysed, or with varying degrees of lysis with an overall average of majority of the spirochetes lysed. In addition, the studies were done, by necessity, with established strains of *B. burgdorferi* s.l. One does not expect all or none phenomena in biology (we should not expect 100% lysis), but even the survival of 1% of spirochetes in a strain that has likely lost much of its variability via isolation and maintenance in BSK medium implies that the original strain in nature might have sufficient diversity for a variant to be selected. Experiments that serially passage surviving spirochetes in deer serum, or the use of representative sets of primary *B. burgdorferi* s.l. isolates, for example, might demonstrate such a possibility. The point is that this theory, based on *in vitro* cultivated isolates and general consistency with field observations, needs to be reexamined in the light of a dynamic environment in which natural selection is constantly operating.

*Host bloodmeal remnant analysis: the key to analyzing host associations*

To date, our understanding of the enzootic cycle has relied on indirect measures such as estimated host abundance, degree of tick infestation, and infectivity to ticks. Mosquito biologists have long used the forage ratio, the proportion of bloodmeals from a host relative to the proportion that host comprises relative to all possible hosts in the site where the sampling was undertaken (Hess et al., 1968) to determine whether a mosquito species prefers a host or is opportunistic. Given their wide host range, measurement of the forage ratio for the ticks that serve as vectors for *B. burgdorferi* s.l. is not straightforward. Trappability differs for individual animals within a species and also between species. Small mammal censuses using standard Sherman or Longworth traps tend to bias the results towards small rodents, such as mice, while squirrels, chipmunks, and shrews are more efficiently trapped with other kinds of traps (Huggins and Gee, 1995; Maddock, 1992; Stephens and Anderson, 2014). Birds are rarely concurrently sampled. There have been few comprehensive attempts to evaluate the reservoir capacity of all the possible hosts within a *B. burgdorferi* enzootic site; the most detailed of such studies (Brunner et al., 2008; LoGiudice et al., 2003) have required extrapolation from the literature or modeling to compensate for not being able to sample all kinds of animals. The logistical issues are large. Although one might own and easily set hundreds of mouse traps in one afternoon, few have and can set more than a few dozen larger box traps to sample

**Table 1. Reports of bloodmeal analyses on host-seeking *Ixodes ricinus*.**

| Study | Method | Sensitivity | No. ticks identified | Proportion feeding on Rodents (mice/voles) | Most common host identified |
|---|---|---|---|---|---|
| Kirstein and Gray, 1999 | RLB-cytb | 63% | 50 | 6% | deer |
| Pichon et al., 2003 | RLB- 18S | 53% | 26 | 8% | bird, ruminant |
| Estrada-Peña et al., 2005 | RLB-18S | 41% | 25 | 8% | bird |
| Pichon et al., 2005 | RLB- 18S | 49% | 159 | 5% | songbirds, ruminants |
| Cadenas et al., 2007 | RLB-12S | 44% | 712 | 8% | artiodactyls, red squirrel, boar |
| Humair et al., 2007 | RLB-12S | 50% | 53 | 4% | red squirrel, deer |
| Wodecka et al., 2014 | nested PCR-12S | 63% | 327 | 9% | roe and red deer boar |
| Collini et al., 2015 | real-time PCR with HRM d-loop | 65% | 34 | 9% | domestic dog, deer |
| Collini et al., 2016 | Real time PCR with HRM | 55% | 239 | 29% | rodents,dogs |
| Wodecka and Skotarczak, 2016 | Nested PCR with RE digest-12S | 65% | 210 | 8% | boar, fox, red and red deer |
| Honig et al., 2017 | RLB-12S | 61% | 219 | 28% | artiodactyls, rodents |

raccoons. Tending mist nets requires continuous attendance to remove captures and depends on careful selection of the net sites. It would be almost impossible to conduct a longitudinal study of tick and spirochete dynamics within the entire fauna of a site, let alone over several sites. However, the use of game cameras allowed analysis of the possible contribution of medium sized and large mammals in 19 Dutch study sites (Takumi et al., 2019)and, combined with estimates of infection from host seeking nymphs, provided a good picture of the likely main contributors to *B. burgdorferi* BRN there.

Given the issues with estimating the complete diversity and relative abundance of possible hosts within the site and to sample often enough to describe temporal variation in host demography (particularly for larger mammals) within any site, host indices are more commonly used. In malarial entomology, the human blood index (HBI), the proportion of bloodmeals taken from humans by a mosquito population (Garrett-Jones, 1964), was used to demonstrate the effect of spraying DDT or dieldrin on the endophilic cohort of *Anopheles gambiae*, among other uses. Blood meal analysis provides a means to directly identify the host upon which a vector fed. This technique has been successfully utilized for mosquitoes since the 1950s (Weitz, 1956). Sufficient protein remains in the mosquito gut after digestion that hosts may be identified by immunological means. Bloodmeal analysis has proven to be much more difficult for ticks, due to their longer life cycle and complete intracellular digestion of their blood meal. Deer ticks feeding as larvae in August will not emerge as host-seeking nymphs until June the next year; what remains of the blood meal then is almost a full year old and little has survived the physiologic processes of molting.

The reported success of host bloodmeal remnant analysis in field-collected ticks has been variable, averaging about 50% success for tick samples, thus making the data difficult to interpret (Table 1): if the remaining samples had yielded identifications, would the distribution of host prevalences differ? Or is the failure to amplify from a large proportion of hosts because a host was not anticipated in a site and the correct assay was not used? Most of the assays have been based on amplifying mammalian mitochondrial gene sequences using conserved primers, and then identifying the host by reverse line blot using species-specific probes or by melting temperatures of the amplicon. To date, studies utilizing host bloodmeal remnant analysis on known Lyme disease vectors have been conducted only on *I. ricinus* in European sites (Table 1).

These valuable studies demonstrate that, for most of the sites examined, rodents play a very minor role as hosts of *I. ricinus*. Instead, ticks fed on roe and red deer, wild boar, and birds. Only Cadenas et al attempted to identify the blood meal host in a large number (>1300) of ticks (Cadenas et al., 2007). Their data clearly indicate that the majority of ticks in their sites fed on wild boar, deer and red squirrels. However, their efforts to incriminate reservoir hosts

was unsuccessful due to low sample size; only 19% of all ticks tested positive for borreliae; half had successful bloodmeal identification, divided over 2 field sites, while there were fewer than 15 identified ticks for each *B. burgdorferi* s..l. genospecies. Only *B. afzelii* had a significant number of host-identified ticks, and these had fed on virtually every type of host for which assays were available; this finding is inconsistent with the current paradigm that this genospecies is maintained by voles. Of course, for those studies analyzing adult ticks, infection could have been acquired as a larva, but evidence of the identity of the infecting host would have not been retained into the adult stage.

Our laboratory is currently refining a new DNA-based method for blood meal remnant analysis that has the potential to overcome the sensitivity issues observed to date. Retrotransposons are genetic elements that integrate into the genome and can replicate themselves, leaving a copy behind in the genome after every jump. The most common forms are LINEs (long interspersed repetitive elements) and SINEs (short interspersed repetitive elements). As they have an ancient origin, over time they have accumulated extremely high copy numbers and have coevolved with their host genomes creating family-specific lineages. Previously called junk DNA, they are thought to make-up 40%of the human genome and similarly large portions of many other mammalian genomes. This extremely high copy number maximizes the probability that a fragment that can be targeted for amplification by PCR will remain after digestion in the tick gut. To date, infection status (using a multiplex real time PCR for *B. burgdorferi*, *B. microti*, and *Anaplasma phagocytophilum*) and host identity can be assayed from single host seeking nymphal *I.dammini.* In our Nantucket field site, during 2018, all the host seeking nymphs that were assayed had fed on *P. leucopus*, but in 2019 only 20% had done so. Interestingly, no change in prevalence of *B. burgdorferi* infection was observed between the years, confirming that even in insular sites, diverse animals may maintain the enzootic cycle. Even more surprising was that in two mainland sites in the same years, *P. leucopus* served as infection source in one while an as yet unidentified host was evident in the other (Goethert et al., submitted). As host bloodmeal remnant analysis is more widely used, we expect to see significant spatiotemporal differences in host contributions to infected ticks, perhaps even to the point that we can no longer say that there is a “main” reservoir host at a scale larger than the specific study site.

Ultimately, we seek to measure the *Peromyscus* blood index (PBI) or that of other species derived from host bloodmeal remnant analysis as a means of comparing the infected vs. noninfected tick samples from sites. An approximate forage ratio may thus be calculated to determine the contribution of mice relative to that of all other hosts identified as sources of infection from the same collection (approximated forage ratio = PBI divided by proportion of bloodmeals from all other hosts; if >1, mice contribute more infected ticks, if 1 then their contribution is no greater than all other hosts; and <1, other hosts contribute more to the force of transmission). Direct measurement of host contributions from host seeking ticks would eliminate the need for time intensive correlative trapping studies combined with xenodiagnosis.

**Factors that influence the abundance of *B. burgdorferi* s.l.**

The classical biotic and abiotic factors that affect abundance of animals and plants are not easily translated to the borreliae-tick-vertebrate system (Figure 4). Such factors would largely affect the hosts that are infected (vertebrate and tick), but it is possible that there are effects that directly bear on the spirochete. With respect to climate, a major abiotic factor, there is no question that temperature impacts the perpetuation of *B. burgdorferi* s.l., mainly with effects on the development and behavior of the tick, but also likely on its microbiota. For example, *B. burgdorferi* survives longer in ticks held in the laboratory at 21ºC vs. those held at 26ºC (Shih et al., 1995), suggesting infection stability is temperature dependent. The quantum of spirochetal infection within a tick may be associated with the latitude where the tick is found, perhaps by direct effect of temperature on spirochetal replication within the tick after molting (Sirotkin and Korenberg, 2018). It is also possible that borreliae become senescent faster if temperatures are warmer while the tick is seeking hosts. It is possible that temperature could act on tick innate immunity or its microbiome, which in turn would act on the spirochete, instead of directly on it.

Temperature, particularly in association with relative humidity (ticks are affected by “saturation deficit”, a combined temperature and relative humidity index below which their activity and survival are curtailed), may be the most influential of the factors affecting

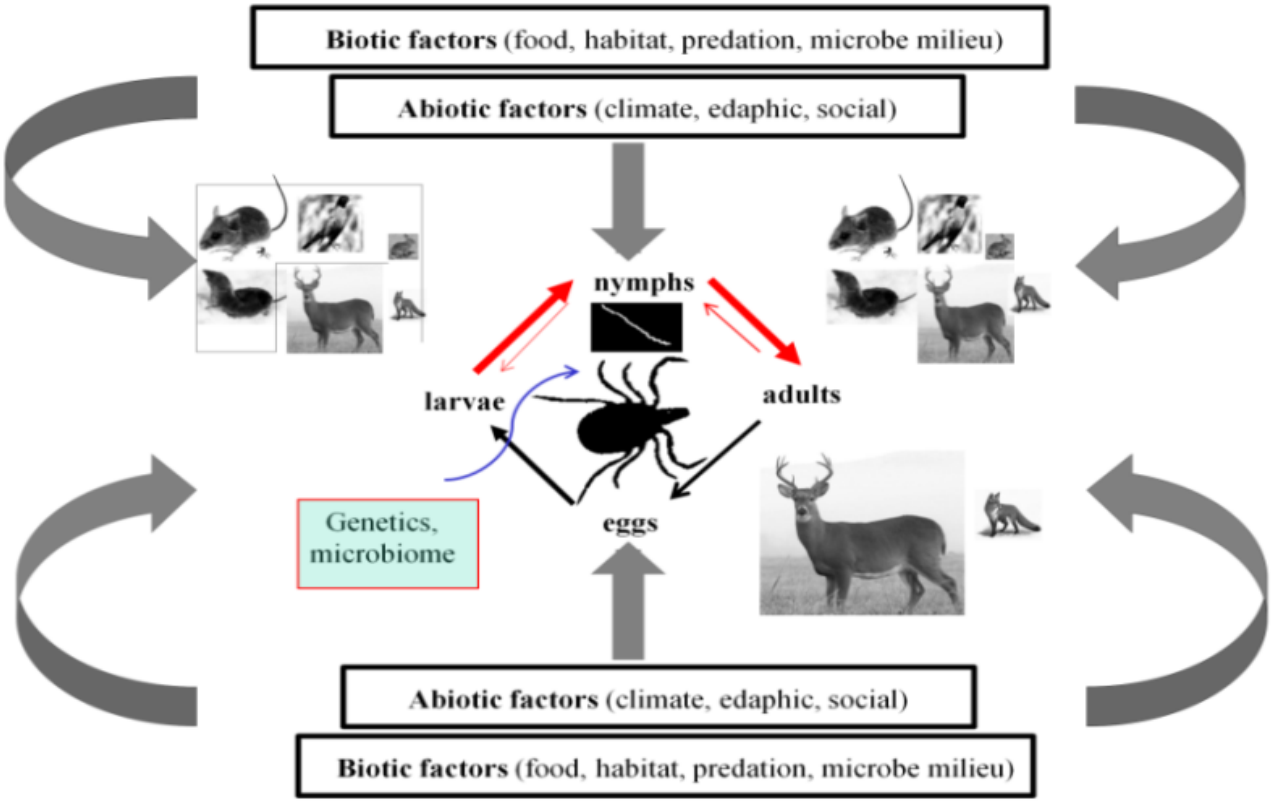


**Figure 4.** General factors operating on enzootic transmission of *B. burgdorferi.* Red arrow = flow of infection.

developmental rates as well as host seeking behavior and longevity in tick populations. Near ground temperatures, day length, and relative humidity are good predictors for nymphal *I. ricinus* activity (Daniel et al., 2015). Host seeking tick abundance, as measured by drag sampling, is reduced by the number of adverse moisture events (Berger et al., 2014; Perret et al., 2000) that is, periods of >8 hours with relative humidity <82% within the leaf litter within a transmission season. In our sites in coastal Massachusetts, the seasonal activity of nymphal *I. dammini* terminates in mid-July, coincident with mid-summer drought conditions, but when conditions remain cool and wet, nymphs may seek hosts well into August. Extreme variability of weather (*e.g.*, episodes of dryness) was associated with a decline in host seeking nymphal *I. ricinus* density, as opposed to overall temperature trends over a 15 year observation period (Hauser et al., 2018). Much has been speculated with respect to climate change and risk of tick transmitted infections, but any effects are likely to be locally dependent. The prevalence of infection of *B. burgdorferi* s.l. in host seeking *I. ricinus* seems remarkably stable across a continental scale, on the order of 11-14% in metanalysis studies with no strong evidence for increase or decrease over 30 years (Rauter and Hartung, 2005; Strnad et al., 2017), arguing against a role for climate change on a continental scale (global temperatures have increased during that time). Of course, there may be site-specific longitudinal changes, particularly where tick density changes: prevalence depends on density of ticks (Coipan et al., 2013; Randolph, 2001), with lesser prevalence where tick numbers are unusually great (>100 per square meter).

Lab studies poorly replicate natural conditions in which ticks are found. For example, it should be noted that the average annual temperature for coastal Massachusetts is 9ºC, taking into account daily maxima and minima and seasonal periods of cold and warm. At a constant 9ºC temperature, tick development does not proceed in the laboratory; in nature, development and activity is associated with

accumulation of heating degree days (Ogden et al., 2004; Rand et al., 2004). At 24ºC, engorged larvae may molt to nymphs within 3 weeks, as do engorged nymphs (unpublished); constant temperatures >28-30ºC greatly reduce daily survival even at constant appropriate humidity (unpublished; (Ogden et al., 2004). As with many other facets of the ecology of Lyme disease, such studies examine a snapshot in time: natural selection may modify traits to allow for specific adaptation to a changing environment. For example, in the early 1990s, stable *I. dammini* infestations started to appear in west-central Massachusetts when previously they had only been found within 30km of the coast. The hypothesis at that time was that it was “too cold” to allow for the full life cycle to proceed because those from coastal sites would poorly molt (taking 50% longer with less success) from larvae to nymphs at 15ºC. Ticks from the Quabbin reservoir (west central Massachusetts) would do so without delay. When the Quabbin ticks were mated with ticks from Ipswich (northeastern coast), an "intermediate” molting temperature (one month at 18ºC) was noted for the progeny (unpublished). This single experiment, not pursued further, suggests a genetically based trait upon which selection could act to allow for invasion of colder sites. It follows that upper Midwestern U.S. populations of *I. dammini* might be cold adapted and thus be able to develop at lesser temperatures. Range expansion of *Ixodes* spp. could be due to local selection for lineages that can adapt to differing temperatures, as opposed to being presumed as evidence for climate change’s effects.

Edaphic factors comprise those related to soil, including plant communities that are regulated by and in turn influence soil composition. Early GIS studies suggested that *I. dammini* abundance was associated with well drained sandy soils with a low water table, and negatively associated with wetlands or saturated soils (Glass et al., 1994). Moder or duff mull humus, in which there is accumulation of organic residues due to slow action of litter dwelling animals and fungi, was associated with nymphal *I. ricinus* density (Goldstein et al., 2018). However, in a more comprehensive analysis, macro- and microhabitat drivers determined nymphal *I. ricinus* abundance, with distance between trees and abundance of trees and shrubs serving as influential explanatory variables (Ehrmann et al., 2017). Functional properties of the habitat (e.g. presence of berries) was more influential as a predictor than soil quality. Interestingly, the most influential single driver for nymphal abundance was larval abundance, which suggests that the reproductive hosts for these ticks (larger mammals, mainly deer) are a critical determinant for transmission (nymphs being the pivotal stage of the tick for maintaining *B. burgdorferi* s.l.). An excellent review of the role of edaphic factors in tick ecology (Burtis et al., 2019) makes the astute point that, although most research on ticks focuses on host seeking stages, the majority of the tick life cycle occurs while the tick is sessile (engorged, molting, diapaused, resting) and takes place within the soil or leaf litter.

A “social” component of abiotic factors, comprising landscape disturbance (natural disasters) and anthropogenic factors (sometimes classified in a category of its own, e.g.,(Morris et al., 2020) creates opportunity for range expansion for *B. burgdorferi*-infected ticks. The emergence of Lyme disease in eastern North America was due to reforestation, increased abundance of deer, and suburbanization (Spielman et al., 1985). 100 years ago, many of the northeastern U.S. sites were characterized by a treeless landscape. Abandonment of farms has led to reforestation, with shrubby early to mid-successional habitats giving way to forests on their way to maturity (Foster et al., 1998). Successional forests may differ with respect to fauna and flora, depending on the duration of succession; mature forests may even be less biodiverse than those in early to mid-succession (Brooks et al., 2012). Risk due to *I. persulcatus*-transmitted infections may relate to cycles of timber harvesting in taiga forests (E. Korenberg, personal communication; (Tupikova and Korenberg, 1965)). The taiga is clear cut and development of successional habitat promotes greater densities of ticks, due to increased activity of ruminants (tick reproductive hosts) that browse shrubs as well as activity by people that exposes them to ticks (Korenberg, 1994). Once the taiga is regenerated, the *Borrelia*-tick biocenosis is stable, but people are less exposed because they do not visit within the deep forest as frequently and such sites have fewer ungulates. Such a scenario may characterize the suburban sites that comprise human risk in the eastern U.S., where new suburban developments create early successional habitat that can promote dense tick infestations coincident with those of the generalist rodent invaders such as white footed mice, chipmunks, and squirrels. Deer benefit from the nitrogen rich plantings in suburbia, as well as protection from hunting (which is usually discouraged). Suburban sites are thus prone to

develop dense deer tick infestations due to being plagued by abundant deer, which deposit engorged female ticks. Some of the range expansion of *I.dammini* and risk of Lyme disease in the northeastern and Middle Atlantic states in the past 30 years may relate to increased development; in Massachusetts, 40 acres of land (90% in forested sites) was lost each day between 1985 and 1999 (Breunig, 2003). The majority of the development was for large lot, new residential construction.

Biotic factors include food, habitat, predation, and microbial communities. Of these, habitat and predation would influence ticks directly; microbial communities would influence the spirochete, tick, and vertebrate hosts; and all 4 would influence the vertebrate hosts. The biotic factors influencing vertebrate hosts is beyond the scope of this review. Habitat interacts with temperature and relative humidity as well as host abundance to help determine tick abundance, *vide supra*. Little is known about predation on ticks; other than parasitic chalcid wasps (Collatz et al., 2011; Hu et al., 1993), there are no predators adapted to ticks. Ants, beetles, spiders and other generalist arthropods may opportunistically prey on ticks as may ground foraging birds. None of these are thought to regulate tick abundance. Indeed, on Naushon Island, Massachusetts (which was the site of a chalcid wasp release in 1928, (Larrousse et al., 1928), as many as 40% of the host seeking nymphal *I. dammini* was infected each transmission season by *Ixodiphagus hookeri*, the chalcid wasp, suggesting that the wasp could kill a large fraction of the nymphs without negative effects on the tick population (T.N. Mather, personal communication).

It is likely that stable enzootic cycles may not require many ticks. *B. burgdorferi* was enzootic at least a generation before it became zoonotic in the U.S. given evidence of infected mice from 1896 and ticks from 1942 (Marshall et al., 1994; Persing et al., 1990). At the turn of the 20th century, the northeastern U.S. landscape was largely farmland (Cronon, 1983). Other than small interspersed herds, deer were largely extirpated Kilpatrick and LaBonte, 2007). The emergence of Lyme disease as a public health problem during the latter half of the 20th century coincided with the abandonment of farmland and reforestation; an expansion of deer herds in density and distribution; and suburbanization (Spielman et al., 1985). Deer, in particular, were central to the development of the zoonotic condition because they serve as the main reproductive hosts for *I. dammini* (Wilson et al., 1988), and risk is associated with tick density. However, for perpetuation of *B. burgdorferi*, it seems likely that dense infestations of ticks may not be required. The Great Island deer reduction experiment, which demonstrated causality between deer abundance and that of *I. dammini*, also provides evidence that an 80% reduction in tick density did not alter the seroprevalence of *B. burgdorferi* in white-footed mice, although seroconversion of mice was delayed by a month (Brunet et al., 1995a). The less efficiently transmitted *B.microti*, however, became less prevalent on Great Island among mice and its genetic diversity declined (unpublished). Prior to its emergence as a zoonotic infection, *B. burgdorferi* must have been maintained in the northeastern U.S. in a generally deer-deficient landscape that could be perpetuated in the absence of hosts that allow the production of dense tick infestations.

*Mixed borrelial infections*

The actual dynamics of spirochetal infection pose questions for analyses of BRN. The quantum of infection (density of spirochetes) within ticks is not homogeneous. The estimated quantum within host seeking ticks ranges from a few hundred to tens of thousands of spirochetes per tick, with an average of 3,000 or so for adult ticks (Brunet et al., 1995b; Kovalevskii and Korenberg, 1995). However, individual ticks may contain as many as 20,000 (Burkot et al., 1994). The significance of such heavily infected outliers for BRN is not clear: humans might suffer greater pathology (assuming a dose dependence for disease), but reservoir hosts might be rendered more infectious to ticks or perhaps a greater inoculum might induce more rapid immune selection. The routine use of ultra-sensitive PCR assays also confounds parameterizing and validating models for BRN. The infectious dose for a mouse is thought to be on the order of a few dozen to a hundred spirochetes (Wang et al., 2003; Kern et al., 2011). Would a tick with a spirochete burden at the limit of detection have any relevance to BRN?

*B. burgdorferi* s.l. may exist only rarely nature as a single clone within a reservoir or tick (Nakao and Miyamoto, 1995; Postic et al., 1998; Qiu et al., 2002; Pérez et al., 2011). When two different strains of *B. burgdorferi* s.s. infected white footed mice, only one of the two was efficiently transmitted to xenodiagnostic ticks (Derdáková et al., 2004). Competition between *B.afzelii* strains manifested as reduced efficiency of infection of xenodiagnostic ticks

as well as quantum of infection for either compared to single infections (Genné et al., 2018, 2019).The average quantum of infection for individual strains diminished as strain richness increased (Strandh and Råberg, 2015), suggesting that competition may act to limit the overall spirochetal burden to the tick (Durand et al., 2017). However, those strains that achieve greater infection intensity in either the reservoir or vector are more likely to be transmitted (Rego et al., 2014). The contribution of those "lesser" strains to BRN requires further analyses: although apparently less fit, nonetheless they exist in natural populations. Balancing selection may maintain such strains; they serve as variation upon which selection may act in a changing environment (new hosts or abiotic influences), ensuring BRN >1 over time.

*Does infection influence the fitness of its hosts?*
White footed mice infected experimentally with *B. burgdorferi* s.s. generally failed to demonstrate pathology (Wright and Nielson, 1990; Schwanz et al., 2011), although young mice sustained carditis and multifocal arthritis (Moody et al., 1994). Infected *P. leucopus* had 20% more larvae infesting them than non-infected mice (Ostfeld et al., 2018), suggesting that infection altered grooming efforts. Infection did not influence mouse survival in a capture mark release study (Voordouw et al., 2015). With *B. afzelii*, infection did not influence survival of *Myodes glareolus* (bank vole), a main host for this genospecies, and although infection did not affect reproductive success of female voles, males were less likely to pass on their genes (Cayol et al., 2018). Kittiwakes infected by *B. burgdorferi* s.l., presumably *B. garinii*, did not differ in survival rates from those treated with an antibiotic (Chambert et al., 2012). *B. burgdorferi* s.l. does not appreciably alter the fitness of its vertebrate host, which may have promoted its ecological success across the Holarctic. Additional analyses might focus on whether very young animals might be burdened given the carditis and arthritis that have been reported; recruitment influences host population density and demography. There is some data that *B. burgdorferi* s.l. may actually enhance the fitness of its vector: infected ticks were more capable of surviving saturation deficit than those that were not (Herrmann and Gern, 2010).

*Concurrent infections*
Like all animals, ticks have a microbiome. A diverse guild of microbial agents may be found in the *Ixodes* spp. that serve as vectors for *B. burgdorferi* s.l. in any site (Carpi et al., 2011; Vayssier-Taussat et al., 2013; Couper et al., 2019; Li et al., 2020). These microbiomes may differ among ticks sampled from diverse sites (Van Treuren et al., 2015) and may differ between developmental stages, with much of the diversity originating from the environment as opposed to the bloodmeal (Zolnik et al., 2016), reflecting the close contact that a tick has with soil and leaf litter. Some reassessment of the reported microbiomes may be required given the possibility of unconsidered procedural artefacts during whole genome analyses (Lejal et al., 2020): about half of the operational taxonomic units (OTUs) identified in host seeking *I. ricinus* could be attributed to contamination of laboratory reagents. Nonetheless, *B. burgdorferi* s.s. less efficiently colonized the guts of subadult *I. dammini* held within sterile containers compared to the same colony held in nonsterile containers (Narasimhan et al., 2014), and the same effect was seen when larvae were allowed to feed on mice treated with gentamicin and infected with a gentamicin resistant strain of *B. burgdorferi* s.s. Dysbiosed ticks engorged better (weighed more) than those that were held in nonsterile conditions or not treated with gentamicin but were less likely to be infected, suggesting that perturbation of the microbiota modified vector competence. Accordingly, differences in prevalence of infection as well as individual quanta among host seeking ticks may need interpreting in the context of the microbial environment. There are few reports of whether members of typical small mammal parasite guilds may influence the reservoir competence for *B. burgdorferi* s.l., but there may only be rare effects given that all animals in nature are concurrently infected by diverse viruses, protozoa, helminths, and ectoparasites ("polymicrobial"). For example, the effect of *Heligmosomoides* sp. (a common nematode infection of wild rodents) was analyzed by experimental infection. Nematode infection along with *I.ricinus* subadult infestation led to greater Th2 responses in a mouse model, but this did not affect susceptibility to *B.afzelii* (Maaz et al., 2016).

The "coinfecting" agents that have received the most attention are ones that may infect and cause disease in humans, viz. *B. miyamotoi*, *B. microti*, *B. divergens* species complex, *Anaplasma phagocytophilum*, *Ehrlichia muris*, and the tickborne encephalitis complex of viruses, among others. Although "coinfection" with *B. microti* may modify the course of Lyme disease (Krause et al., 1996), the ecological effects of concurrent or sequential infection with the zoonotic ones remain debatable, particularly when

one considers that wild animals and ticks contain diverse microbiota that may be more influential than those that we focus on because of their zoonotic impact. Concurrent infection by *B. microti* and *B. burgdorferi* did not appreciably change the transmission efficiency of spirochetes in an experimental *P. leucopus* model (Mather et al., 1990). However, *B. burgdorferi* s.s. appears to alter the susceptibility and infectivity of *P. leucopus* for *B. microti* and *A. phagocytophilum* (Dunn et al., 2014; Levin and Fish, 2001). Trying to determine whether there are fitness effects of concurrent infection within naturally infected ticks (analyzing host-seeking ticks) would be confounded: if there were fewer borreliae within a tick that also contained *B. microti*, is this because the host upon which the larva fed had few spirochetes or is it because there was some inhibitory influence of *B. microti* on the spirochete within the tick? Analyzing the naturally infected vertebrate hosts is equally difficult to interpret because of the complex mix of variables (immunogenetics, age, maternal effect, nutritional status).

*Factors that influence the distribution of* B. burgdorferi *s.l.*

What limits the distribution of the borreliae-tick-vertebrate system? *B. burgdorferi* s.l. are found on all continents except Antarctica, Australia, and Africa (enzootic cycles are present in North Africa). Relatively recent transport by humans of infected hosts, e.g., by livestock or rats (Matuschka et al., 1996; Smith et al., 1993), is possible; *R. norvegicus* is reservoir competent for *B. burgdorferi* and can maintain the enzootic cycle. Mammals and their ectoparasites could have moved North to South (or perhaps the other way) during the Great American Biotic Interchange (Panamanian isthmus) in the late Miocene through Pleistocene. *I. scapularis* infests deer in Panama (Fairchild, G.B. et al., 1966). It is also possible that mammals introduced infection during the several Beringian migrations during the last glaciations (Goethert et al., 2006). The main explanation, though, may be the transport of ticks or infection by birds. The pelagic bird tick *I. uriae* maintains *B. garinii* across the world, and even in colder sites in the northern (Iceland, Bering Straits) and southern (Crozet Islands, Falkland Islands) hemispheres (Olsen et al., 1995), demonstrating the importance of bird transport. *B. burgdorferi* has greatly extended its range across the eastern U.S., with strong evidence for the role of migratory birds in transporting ticks, as well as infection (within ticks and with birds themselves being reservoir competent) (Battaly et al., 1987; Ogden et al., 2008; Smith et al., 1996). In fact, *B. afzelii* reactivates in birds that are stressed from migration (Gylfe et al., 2000), suggesting a mechanism for long distance transport of infection. In a recent comprehensive European study, 37% of over 2000 ticks removed from more than 800 birds of 28 species were infected by *B. burgdorferi* s.l., demonstrating not only their critical role in dispersal of the bacterium but also, interestingly, their role in spatial mixing of spirochetal genotypes. The rodent-associated genospecies, in contrast seem less diverse and more likely to be geographically restricted (Norte et al., 2020).

The evidence for enzootic *B. burgdorferi* s.l. in tropical sites includes that from Taiwan and southern mainland China, as well as the southern cone countries of South America. These mainly comprise associations of newly recognized genospecies and *Ixodes* spp. that are generally rodent-infesting but also will attach to humans (e.g., *B. valaisiana* and *I. granulatus* in Taiwan, (Chao et al., 2010); *B.yangtze* and *I. granulatus* in southwestern China, (Chu et al., 2008); *B. chilensis* and *I. stilesi* (Ivanova et al., 2014); *Borrelia* sp. and *I. pararicinus* (SarachoBottero et al., 2017). The influence of temperature on the stability of vector infection by the diverse *B. burgdorferi* s.l. may be an interesting focus of future inquiry, given that most attention has been placed on the *B. burgdorferi* s.l. of northern latitudes, which is less likely to persist in the vector at greater temperatures (Shih et al., 1995).

*The ecology of* B. burgdorferi *s.l.: a synopsis*

Although there is a main theme in zoonotic sites (*I.ricinus*/*persulcatus* group ticks feeding on small mammals and birds maintain the enzootic cycle), the complexity that is evident across its range cannot simply be characterized as a range of performances of a symphonic piece by a range of instruments from kazoo (Massachusetts islands) to full orchestra (central Europe). The adaptive plasticity of the spirochete and tick has generated unusual enzootic cycles such as *B. lusitaniae*, *I. inopinatus*, and sand lizards; *B. garinii*, *I. uriae*, and pelagic seabirds; and *B. californiae*, *I. jellisoni* and kangaroo rats, among others. These have developed into distinct symphonies, and it should not be unexpected when other interesting *Borrelia*-*Ixodes*-vertebrate host combinations are reported.

**Soft tick transmitted relapsing fever spirochetes**

Although as diverse as *B. burgdorferi* s.l., the relapsing fever borreliae have one general enzootic cycle, comprising that of nest dwelling (nidicolous), cryptic soft ticks and the small mammals that they feed on.There are, however, some variations on the general theme. In addition, there are two exceptions to the variations on a theme characterization: louse borne relapsing fever, and hard tick transmitted relapsing fever borreliae.

*Diversity of tick-relapsing fever group borreliae associations.*

The systematics of the soft ticks have recently been revised, with two major groupings being recognized, the Argasinae and the Ornithodorinae (Mans et al., 2019). Relapsing fever spirochetes are mainly maintained by ticks of the Ornithodorinae (bold type indicates genera/subgenera including a known vector), although this may reflect the research effort related to human or veterinary health. The Argasinae comprise the genera *Alveonasus*, ***Argas*** (includingthe subgenus *Persicargas*), *Navis*, *Ogadenus*, *Proknekalia* and *Secretargas*. The Ornithodorinae include *Carios* (including the subgenera *Alectorobius, Antricola,* ***Carios****, Nothoaspis, Parantricola, Reticulinasus, Subparmatus*), *Chiropterargas*, *Ornithodoros* (subgenera *Microargas, Ornamentum,* ***Ornithodoros****,* ***Pavlovskyella****, Theriodoros*) and *Otobius*. Many of these genera are monotypic or comprise a small number of species that have very specialized host associations. On the one hand, such specialization does not lend itself to host switches, a main mode of diversification for microbial parasites, but, the focused feeding on unique hosts promotes vectorial capacity and perpetuation if a microbial agent becomes transmitted during bloodfeeding. It seems likely that novel borreliae-soft tick associations will be recognized when more of these ticks (particularly with adequate sampling) are analyzed.

Felsenfeld (1965, 1971) listed 11 Old World (includes *B. anserina*) and 6 New World species of soft-tick transmitted *Borrelia* spp., along with 6 others that he felt were either too incompletely described to warrant recognition or those that he considered as belonging to another valid species. Today, 16 Old World and 8 New World soft tick transmitted *Borrelia* spp. are recognized (Talagrand-Reboul et al., 2018). Whole genome analyses support the classical Old World/ New World dichotomy but suggest some diversity simply represents ecotypes of a single genospecies (Elbir et al., 2014). As with other parasitic organisms (e.g., *Babesia* spp. or trypanosomes), a “one vector-one species” concept influenced the early taxonomy of the soft tick-transmitted borreliae (Davis, 1942) and thus a spirochete detected in a newly examined species of tick was assumed to be novel and worthy of a name. Field collected ticks from diverse Western U.S. sites transmitted spirochetes by feeding on mice. Only colony derived ticks of the species from which an infection originated (*O. turicata*, *O. hersmii*, and *O. parkeri* were fed at the same time) became infected by those spirochetes. The specificity of these associations remain to be fully analyzed, although a simple experiment suggests that there may be a salivary gland barrier: *O. turicata* and *O. parkeri* became infected (*B. hermsii* colonized the gut and disseminated to the hemolymph of both these species) but failed to be transmitted by bite whereas with the homologous association *B. hermsii* was efficiently transmitted by *O. hermsii* (Schwan, 1996). The barrier, thus, may not be just the capacity for spirochetes to attach to and invade the salivary glands, but could involve the salivary pharmacopeia and its influence at the site of inoculation (Krishnavajhala et al., 2018). Some borreliae, such as *B. duttonii,* may be transmitted not only by bite (as subadults) but also by contact with fluid from the coxal glands (an excretory organ that facilitates diuresis after a bloodmeal).

Burrows, logs, bird or bat roosts, mammal nests, caves, huts, and cabins are the venues of transmission. In general, the soft tick-transmitted borreliae are perpetuated in small natural foci (Pavlovsky, 1966), which can comprise just a few burrows in a larger area that contains many burrows or one hut in a village. The limited mobility of the soft tick vector (typically nidicolous, or nest dwelling), and its short feeding durations means that transport to new burrows or huts is a rare event. The limited capacity of these ticks to move or be transported, however, is compensated for by their long lives, often years. If transported, there is a great probability that the introduction will persist due to broad feeding preferences and TOT.. Soft ticks have a great capacity to adapt to diverse vertebrate hosts (Mans et al., 2008): critical salivary anti-hemostatic anti-thrombin and anti-platelet aggregation activities are highly conserved between the phylogenetically basal *A. monolakensis* and *Ornithodoros* spp. However, the argasid salivary pharmacopeia appears to be less elegant than that of ixodid ticks; a prominent

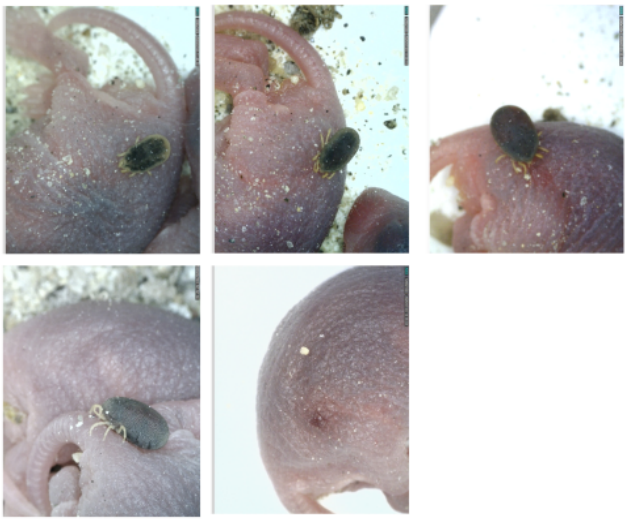

**Figure 5.** *O. hermsii* feeding on suckling mouse over 20 minutes, culminating in hemorrhagic feeding lesion.

hemorrhage or feeding lesion is left behind after many species of soft ticks feed (Figure 5). Then too, inheritance (TOT) tends to be the rule for the soft tick-transmitted borreliae, which suggests the possibility that a single infected fertilized female tick could initiate a natural focus. Thus, although transport to expand range is likely a rare event, when it happens introduction and development of a new soft tick-relapsing fever borreliae focus could be efficient.

As with studies of TOT for other vector-pathogen relationships, published reports of the efficiency of the process range from documenting that it does happen, to a more comprehensive analysis. The transovarial transmission rate (TOTR) is the percentage of infected females that transmit an agent to their progeny, without reference to how many of the progeny are actually infected. The filial infection rate (FIR) refers to the proportion of an infected female's progeny that are actually infected. The product of the FIR and TOTR is the vertical transmission rate (VTR), which is a measure of population prevalence and thus a more useful estimator for models of BRN (TOTR could simply be based on a few ticks that laid a few infected eggs, which might not survive to contribute to a new generation of infected ticks). There are very few reports that present data to estimate the VTR for the relapsing fever group borreliae.

*Factors that influence the distribution of relapsing fever borreliae.*

The ecology of soft ticks is not easily studied given their cryptic niche. They occupy crevices and burrows and are found under logs, rocks and bark; they are nocturnal or at least are active only under reduced light conditions. Few species have extended feeding durations and thus most are not to be collected from hosts. The standard methods for collecting hard ticks (dragging or flagging; trapping and examining vertebrate hosts) are not relevant. Some species, such as *O. coriaceus*, may be attracted by carbon dioxide and will accumulate nearby, to be collected by hand. Sentinel animals may be placed near potential tick hiding places and inspected frequently to find feeding ticks (Adeyeye and Butler, 1989). Most sampling methods comprise digging out or vacuuming a burrow and sifting the soil to look for ticks (soft ticks fluoresce under ultraviolet light, so sand collections may be easily screened by the use of a Woods lamp in a darkened room). Crevices or other hiding places within a house may be visually inspected and ticks collected with forceps. In short, the effort required for studying soft ticks is much more than for most hard ticks, leading to a paucity of published studies of their ecology. Surveys that serve as the basis for distribution maps tend to be biased by the inclusion of sites that come to our attention because of investigation of reports of human infection, which reflects zoonotic spillover due to local tick abundance; intense human exposureto sites where soft ticks are common; or investigator interest (e.g., the many contributions of the Rocky Mountain Laboratories to relapsing fever in the western U.S.).

A recent analysis using species distribution modeling of environmental variables suggests that the range of *O. turicata* (and potentially *B. turicatae*) in the U.S. is likely underestimated (Donaldson et al., 2016). Such an approach may allow for more targeted sampling efforts to fill in distributional gaps. Indeed, the distributional modeling results indicated possible overlap in the distributions *O. turicata* and *O. talaje*, and, in fact, the same woodrat and burrowing owl nests in central and southern Texas have yielded both species (Lopez et al., 2016). How the two species can occupy the same niche remains to be described.

One of the few long-term studies of the ecology of borreliae in relation to human risk for relapsing fever provides some general observations about their

**Figure 6.** *O. tartakovskyi* feeding on suckling mouse. All stages may feed simultaneously but may differ in feeding duration on a host.

distribution. Cases spatially clustered in southwestern Senegal near homes where *O. sonrai* had been collected and were most common in March(Vial et al., 2006a). Most cases were diagnosed about 4 months before the rainy season, not because the ticks were affected by the external conditions within their cryptic resting sites, but because small mammal populations were at their lowest abundance during the end of the dry season (Talagrand-Reboul et al., 2018). When small mammals are available, the ticks are more likely to feed on them, but when their density is low, humans become the source of bloodmeals. This pattern of small mammal scarcity (due to either seasonality or other factors leading to local declines) serving to focus bites on humans is seen with the paradigm of relapsing fever in the western U.S., where cabins left empty for the winter are suddenly occupied by human visitors (e.g., the Grand Canyon outbreaks (Paul et al., 2002). In such vacation cabins, rodents that nested there during the winter move to outdoor sites during the spring, leaving the long-lived ticks behind to attack new occupants (Wynns, 1942). The relevance of these epidemiological observations is that there is strong spatial clustering of transmission and the enzootic cycle is typically cryptic unless the small mammal hosts become scarce. This is as expected from the theory of natural focality, in which infectious agents such as the soft tick transmitted borreliae, generally exist in “nidi” or nests such as the burrows of rodents, which are “biocenoses” with optimal microhabitat and temperatures (Pavlovsky, 1966). Such nidi are relatively isolated from each other if the vector tick has limited mobility (due to rapid feeding, not being transported from a burrow) and humans never see the tick or infection unless they trespass into the nidus.

At the level of the deme, soft ticks generally have limited capacity to move between burrows or other nidi, although this is influenced by the duration of bloodfeeding attachment that characterizes a species. All stages (larvae, several nymphal stages, adults) are found in the same burrow (Figure 6). A capture-mark-release study of *O. turicata americana* in Florida demonstrated that marked ticks were never found to move from the gopher tortoise burrows into which they were released (Adeyeye and Butler, 1989); burrows within visual distance in a site could remain completely uninfested and in fact in one of the two study sites only 4 of 10 burrows contained ticks. *O. maritimus* infesting yellow-legged gulls, however, appeared to be able to move and recolonize empty nests, a conclusion based on microbial prevalence, which did not demonstrate spatial structure within the breeding site (Dupraz et al., 2017). Only 0.9% of marked *O. coriaceus* were found 15m away from where they were released (Garcia, 1963). At a regional scale, populations of *O. coriaceus* across California, Oregon and Nevada were highly structured with little evidence of gene flow as determined by mtDNA analysis (Teglas et al., 2005), suggesting that this tick has limited dispersal capacity despite the fact that its larvae may attach for many days to its highly vagile deer host. *O. sonrai* in Mauritania and Senegal demonstrated large diversity in 16S rDNA sequences, with 4 major subclades being detected; although there was some geographical clustering of subclades, in general genetic divergence did not correlate with increasing

geographical distance and hence was interpreted as being inconsistent with the hypothesis that isolation is a function of low dispersal capacity (Vial et al., 2006b). Nonetheless, the main pattern of distribution of soft ticks is exemplified by long term micronidi that are determined by animal host resting behavior and microhabitat.

*Fitness effects of infection*

There is some evidence that the vertebrate hosts infested by soft ticks may be affected by tick feeding, but the influence of spirochetal infection on fitness remains poorly studied. Balearic storm petrel nestling survival was less in deeper aspects of the breeding cave where *O. maritimus* were dense, and individual nestling body weight was reduced for those with greater tick burdens (Sanz-Aguilar et al., 2020). Dense infestations of chickens by *A. persicus* reduce their egg laying capacity (Khan et al., 2001) and acute infections of fowl spirochetosis (*B. anserina*) can cause mortality. Sensitization of hosts by repeated feeding reduced *O. turicata* engorgement weights (Need and Butler, 1991), and fecundity of *O. moubata* was reduced (Chinzei and Minoura, 1988), suggesting possibilities for density dependent modulation of tick populations. Although *B. turicata* infected ticks remained infectious after 5 years of starvation (Francis, 1942), demonstrating remarkable longevity of both tick and spirochetes and general lack of effect on fitness, the study did not determine whether uninfected ticks could have lived even longer.

A poorly studied phenomenon, interference, could in some instances enhance the fitness of a vertebrate host that is infected by relapsing fever group borreliae. Trautman (Trautman, 1907) demonstrated that co-inoculation of a spirochete, presumably *B. duttonii*, influenced the parasitemia and disease due to *Trypanosoma vivax* and *T. gambiense* in experimentally infected mice. The effects were not observed when killed spirochetes were administered. Subsequent studies confirmed these results using other trypanosomes, but no effects were seen with plasmodia, rickettsiae, or leptospira. Trautmann did not specifically analyze the effects of trypanosome infection on borreliosis but statedhe had no doubt there was “egalement une action sur les spirilles”. The generality of interference has been demonstrated by sporadic reports, for example, interference of a mouse bartonella by a hemoplasma (Eperythrozoon) was subsequently demonstrated by Tyzzer (Tyzzer, 1941), with the former disappearing from circulation when the latter was detected. This poorly studied phenomenon may have implications for the perpetuation of relapsing fever borreliae given the ubiquity of trypanosomes as vertebrate parasites.

*East Africa and endemic tick borne relapsing fever*

Obermeier found “a most delicate and peculiarly motile spirillum in the blood of relapsing fever patients”, seen in samples taken and examined microscopically at bedside in Virchow’s Berlin clinic in 1867. Berlin had been experiencing an outbreak of what is now known as epidemic or louse borne relapsing fever (Birkhaug, 1942). The discovery of the cause of endemic relapsing fever (African tick borne relapsing fever, ATBRF) is a story from the times of colonial exploration, with David Livingstone noting in 1857 that people bitten by ticks in Angola and Mozambique sustained an irregular fever (Geigy, 1968a).The vector of *B. duttoni*, the eyeless tampan *O. moubata* was identified in 1904 by Joseph E. Dutton who with John L. Todd were able to infect monkeys with relapsing fever by the bites of soft ticks; Dutton and Todd both became infected during the course of autopsies of relapsing fever fatalities and Dutton died of the disease. Robert Koch, at the same time, independently infected monkeys by the bites of soft ticks. It is not known whether any of these investigators were aware of the report (Marchaux and Salimbeni, 1903) demonstrating that *Argas miniatus* transmitted fowl spirochetosis.

It is possible, though, that the local inhabitants of East Africa recognized cause and effect long before scientific inquiry, as suggested by Geigy’s (Geigy, 1968a) anecdote about soft ticks being carried by native travelers: “The author of this chapter can confirm this observation from personal experience. In 1957 in Tanzania, he met some native laborers who had come from Rwanda to work on a sisal estate near Morogoro. They carried with them *O. moubata* ticks from their own huts that they kept in matchboxes and allowed them to feed regularly on their skin…they explained that they did so in order not to contract relapsing fever on returning to their homes in Rwanda after a fairly long absence. They obviously knew from experience that a certain degree of protection against their local strain of borreliae could be achieved in this way. Unfortunately, the author could not find out whether their ticks were really infected or not because they refused to hand over these precious creatures.” Thus, the distribution of *O. moubata* could have been heavily influenced by human transport. On the other hand, Geigy

discounted human transport as a factor in the distribution of *O. moubata* and endemic tick borne relapsing fever, and speculated that this tick adapted from inhabiting the burrows of warthog and aardvark to human dwellings because "infested huts are practically always found where warthogs also occur and are hunted" and "conditions in a mud hut are strikingly similar to those in an aardvark burrow".

*O. moubata* is considered a superspecies or species complex with "races" adapted to wild or human domestic life cycles: to tortoises (*O. compactus*), to porcupines (*O. apertus*), and two races (*O. porcinus* and *O. domesticus*) found with warthogs and domestic pigs, respectively (Walton, 1962). The aardvark may be the original host for the *O. moubata* complex given that warthog abundance is apparently associated with that of aardvark burrows (Whittington-Jones et al., 2011). The great interchange of animals between burrows may have led to specific adaptations by the tick (*e.g.*, extended feeding on poikilothermic tortoises). The most parsimonious hypothesis to explain the apparent anthropophily of *O. moubata* sensu stricto is that the proximity of humans and their pigs to warthogs has selected tick lineages that adapted to living within huts, and were subsequently transported by humans, either incidentally or intentionally(Heisch and Grainger, 1950).

Although *O. moubata* has a wide host range, feeding on tortoises, birds (including domestic chickens), aardvark, porcupine, and warthogs or domestic pigs, ATBRF is not a zoonosis, but an anthroponosis, with humans as an amplification host. The classification of ATBRF as an anthroponosis rests on a general failure over the first half of the 20$^{th}$ century to detect *B.duttoni* in any animal other than humans. Experimental studies failed to infect warthogs with laboratory strains of *B. duttonii*; ticks collected from warthog burrows were never found to be infected, and subinoculation of warthog tissue samples into mice failed to demonstrate infection (Geigy, 1968b). This longstanding dogma has been challenged based on PCR detection of *B. duttoni* sequences in chickens and pigs sampled from ATBRF endemic villages (McCall et al., 2007). One might argue that PCR is a significantly more sensitive method to determine infection status of animals, certainly more so than microscopy of blood smears, but the gold standard for older workers was inoculation of diagnostic blood or tissues (often 0.5-1mL volumes) into laboratory rodents and assessing them for evidence of infection. At least with infection, the question of anthroponosis vs. zoonosis bears on interpreting the existing studies of the ecology of ATBRF as well as on planning future studies: a zoonosis is more complicated than is an anthroponosis, particularly to probe for critical BRN factors.

The life cycle of *O. moubata* has been studied by a number of researchers, and was nicely summarized by Hoogstraal (Hoogstraal, 1956). A female may lay as many as 7 egg batches (7 bloodmeals), totaling 500-1200 eggs during its lifetime. The larva that emerges from an egg is nonparasitic and nonmotile and molts to the first nymphal stage, without feeding, within days. Apparently, there is a reddish, hematin-like residue within larvae that may serve as a nutrient, suggesting that elements of the female's bloodmeal have been incorporated during oogenesis. There are 4-8 nymphal instars (a bloodmeal is required for each) before they become adults. The duration of feeding for the nymph ranges from 13-87 minutes, with later instars generally taking longer to feed. The entire life cycle may take as few as 62-73 days in the laboratory. The longevity of an adult tick is usually on the order of a year and a half, but maximal durations of 4-13 years have been reported.

Parthenogenetic colonies of *O. moubata* have been reported, with one well documented report (Davis, 1951) that assured a lack of fertilization by males. Progeny were all female, and all life cycle events (eclosion, molting, oviposition) greatly delayed relative to those that were sexually reproducing. The parthenogenetic females, when mated, produced progeny of both sexes. Whether all populations of *O. moubata* are capable of parthenogenesis remains undescribed. Given efficient inheritance of *B. duttonii*, which may last through 5 generations, albeit with reductions in transovarial transmission rate (Geigy and Aeschlimann, 1964), there is much potential for introduction to new sites as well as maintenance within sites with episodic presence of amplifying hosts.

*O. moubata* is apparently absent from areas with high humidity, although human living conditions may allow for colonization of sites where they would otherwise be excluded. In the laboratory, a saturated atmosphere as well as greater temperature reduces fitness (Cunliffe and Nuttall, 1921). In Kenya, some tribes construct houses with dry raised mud beds, and fires near the beds would dry the ground enough

to promote tick survival (Walton, 1950). Similarly, *O. moubata* may be found in the Transvaal Highveld where there are very cold winters, simply because people have fires within their huts to provide heat (Hoogstraal, 1956). Accordingly, these ticks have interacted with humans to adapt locally and, thus, ATBRF is not only an anthroponosis but also an anthropogenic infection.

*Fowl spirochetosis*

*B. anserina* seems to be restricted to birds, with successful experimental infections of diverse birds but not to rodents or primates (Lisboa et al., 2009; McNeil et al., 1949). Human serum lyses *B. anserina*, helping to explain why zoonotic infection has not been reported even though fowl spirochetosis and *A. persicus* are widely distributed and can readily attain dense infestations within chicken coops, thereby potentially exposing the human caretakers of the birds. Although other *Argas* spp. are known to bite humans, *A. persicus* rarely bite people (Hoogstraal, 1956). The ecology of natural infestations of *A. persicus* and *B.anserina* (as opposed to agricultural settings) is dependent on breeding sites for their hosts, for example, birds that construct large permanent nests near where chickens are actively produced may sustain dense infestations. Heron rookeries near Cairo were found to have tens of thousands of *A. persicus* hiding in crevices and under bark of the fig trees where the herons roosted (Hoogstraal, 1956). Sporadic specimens have been found on diverse birds (e.g., quail, guinea fowl, vultures, cranes) but reports from mammals are rare. Why *B. anserina* has not been reported from wild birds is not known, particularly given the fact that many other *Argas* spp. such as *A. miniatus* are competent vectors (Lisboa et al., 2009).

*A. persicus* larvae have an extended feeding duration of 5-10 days, during which they could be transported; nymphs and adults take fewer than 2 hours to engorge. All feeding is done under reduced light conditions (Hooker et al., 1912). Unfed adults generally live a year, but larvae may survive over 200 days at 21-24ºC and >85% relative humidity, although longevity was reduced to 50 days at 37ºC at the same humidity. One estimate for the vertical transmission rate (VTR) for *B. anserine* was 67% (Zaher et al., 1977). Allowing infested chicken coops to remain uninhabited as a means of eliminating an infestation, therefore, would require an extended duration dependent on temperature. This would imply that peri-domestic infestations (*e.g.*, in a heron rookery) would likelypersist if nests were abandoned for the migratory season. The global distribution of this tick seems limited only by a minimum 20ºC temperature for development. As with other pest infestations of domesticated animals, the increasing availability and use of antiparasitics as well as improved biosecurity and a trend for large-scale poultry production have been reducing the agricultural impact of fowl spirochetosis. *B. anserina* continues to affect poultry production in subsistence settings (Lisboa et al., 2009).

*Borrelia coriaceae and O. coriaceus*

Spirochetes were discovered in *O. coriaceae* during early investigations of Lyme disease in northern California (Lane et al., 1985), differing morphologically and behaviorally from the Lyme borreliosis agent and was described as *B. coriaceae* Johnson et al., 1987). At that time, *B. coriaceae* was considered a likely agent of epizootic bovine abortion, a condition that had long been epidemiologically linked to bites of this tick; the agent is now suggested to be a deltaproteobacterium transmitted by *O. coriaceae*. *O. coriaceae*had previously been demonstrated to be incompetent vectors for relapsing fever borreliae (Herms and Wheeler, 1935) and was known only for its painful bites. The ‘pajaroello’, as it was commonly known (Herms, 1916), was the subject of a comprehensive ecology-focused doctoral dissertation by Garcia (Garcia, 1963), and the following life history details are taken from that source. This is an unusual soft tick that feeds on large mammals such as the black tailed deer as well as cattle along the California coast down into Mexico. Although most feeding is thought to occur when deer or other large mammals bed down under shrubs such as manzanita, these ticks will move up to 25 feet along carbon dioxide gradients to the source. Body heat causes them to accumulate. Unlike most soft ticks, *O. coriaceus* is only weakly negatively phototropic and will move in sunlight. In one of the very few direct observations of predation on ticks, Garcia observed fence lizards (*Sceloporus occidentalis*) eating nymphs and confirmed this by examining gut contents of the lizards. The ticks have a characteristic response to being touched, which was to become immobile with the legs tucked underneath; he speculated that reduces predation by animals that targeted moving prey. The lower temperature limit to eggs eclosing (hatching) is 15ºC and an upper limit 45ºC, with high mortality at higher temperatures and low relative humidity. The larval stage has optimal survival

between 20-25ºC and >70% relative humidity. All stages may be active during all times of the year; rain and low temperatures inhibit activity. Deer are the main bloodmeal sources, and the ticks rapidly feed (an average of 20 or so minutes). Larvae, however, may be associated with the host for longer durations (an average of 9 days) and, thus, could be dispersed to new sites by deer. Coxal fluid is expressed off the host and thus transmission of *B. coriaceae* is via the saliva. Transovarial transmission appears to be inefficient, with a VTR of only 1.5%, indicating the requirement for a vertebrate amplifying host (Lane and Manweiler, 1988); more than half of deer that were sampled during the course of Lyme borreliosis studies in northern California demonstrated spirochetemias, likely due to *B. coriaceae* (Lane and Burgdorfer, 1988). Infection did not affect longevity or fecundity of the ticks (Lane and Manweiler, 1988). Like many soft tick species, adult *O. coriaceus* could survive as long as 5 years (Smith, 1944).

**Hard tick transmitted relapsing fever spirochetes**

Sir Arnold Theiler was the first to demonstrate that a spirochete was transmitted by hard ticks (Theiler, 1902); he demonstrated its presence in cattle during studies of theileriosis. He apparently thought little of the finding, particularly given that there was no pathology attributable to this spirochete (bovine borreliosis comprises fever, anemia, and transient malaise). Laveran named the organism *Spirillum theileri* from slides that Theiler had sent him. Theiler suspected that ticks served as vectors in his original report, noting that the borreliae accompanied piroplasm infections, and subsequently (Theiler, 1905) transmitted this infection to cattle using *Rhipicephalus (Boophilus) decoloratus*. The spirochete must be inherited (TOT) because all 3 motile life stages (larva, nymph, adult) of *R. decoloratus* develop on the same individual animal. Fed female ticks dropped from the host and eggs were laid in the environment; larvae emerged, attached to a new cow, fed to repletion, and molted in situ within a matter of days to the nymphal stage. The nymph, without moving (or moving very little) attached, fed, and developed in situ to the adult stage. Male ticks may move on the host to find a female. Theiler was working on theileriosis and babesiosis and knew about Theobald Smith and Fred Kilborne's pioneering incrimination of *R. annulatus*, a very similar one host cattle tick, as the vector of Texas cattle fever (due to *Babesia bigemina*). Inheritance was critical to perpetuating that parasite and Theiler also found that the borreliae were passed from the infected female through the egg to the larva. TOTR ranged from 70%-80% over 3 generations (Smith et al., 1978), confirming that inheritance is likely to be the main mode of perpetuation. Interestingly, although Theiler stated that larvae were infectious, subsequent work (Callow, 1967; Smith and Rogers, 1998) failed to confirm transmission by larvae. Smith and Rogers argued that even though larvae were infected, as demonstrated by detecting spirochetes within larval tissues, perhaps only nymphs were able to deliver sufficientinocula.

*B.theileri* has since been reported from many parts of Africa, Australia, and South America and undoubtedly has been transported globally with cattle as have their vectors, mainly *R. microplus* and *R. decoloratus*. Sheep, but not goats, horses, dogs or rodents were experimentally infected by blood subinoculation (Theiler, 1902). Deer can be infected by *B. theileri* (Callow, 1967), although spirochetemias are not detectable by microscopy. Evidence of infection was obtained by subinoculating blood into cattle, which became infected.The possibility that deer may become infected is of relevance given that they are naturally infested by *R..annulatus* in Latin America, and thus might serve as wildlife reservoirs for the agent of bovine borreliosis. Thus, although perpetuation of *B. theileri* mainly depends on lack of tick control in domestic animal production, an ancillary enzootic cycle between ticks and deer would perpetuate *B. theileri.* Indeed, in American sites (Texas, Florida) where *R. microplus* and *R. annulatus* were eradicated from cattle by the U.S. Department of Agriculture, infestations continued to appear due to maintenance of these ticks in the wild by deer (Pound et al., 2009). Whether deer maintain *B. theileri* remains to be explored.

Lyme disease-like illnesses of undocumented etiology have been confused with Lyme disease in sites where Lone Star ticks (*Amblyomma americanum*) are found (Campbell et al., 1995; Kirkland et al., 1997). This syndrome, termed Masters' disease (Armstrong et al., 1996) or southern tick-associated rash-illness, STARI, is characterized by an erythema migrans rash from which no evidence of *B. burgdorferi* may be detected, accompanied by generally mild constitutional symptoms with no chronic sequelae. Reactivity to *B. burgdorfer*i antigens seems rare in sera from these patients (Campbell et al., 1995) but see Master and Donnell (Masters and Donnell, 1995). Lone Star ticks are not competent vectors for *B. burgdorferi*

(Piesman et al., 1979; Piesman and Sinsky, 1988) but are infected by *B. lonestari* (Armstrong et al., 1996; Barbour et al., 1996), which are closely related to *B.theileri* (Rich et al., 2001). The hypothesis that remains to be adequately tested is that *B. lonestari* is the etiologic agent of Masters' disease; definitive evidence for or against the hypothesis has not been reported. The prevalence of infection in Lone Star ticks ranges from 0-5% (Armstrong et al., 1996; Barbour et al., 1996; Kocan et al., 1992; Schulze et al., 1986), but given the proportionally greater densities of these ticks in endemic sites, compared with deer ticks, transmission risk indices for *B. burgdorferi* and *B. lonestari* differ only by 50% (Armstrong et al., 1996).

Deer serve as the main hosts for all stages of Lone Star ticks (Hair and Bowman, 1986), although raccoons, opossums, fox, coyote, rabbit, and turkeys are infested by subadult stages. Experimental inoculation of deer with in vitro cultivated *B. lonestari* produced spirochetemia that was detectable by blood smears and PCR (Moyer et al., 2006). About 15% of deer contained *B. lonestari* antibodies as detected by indirect immunofluorescence antibody testing, with a greater proportion seropositive from southern sites than in northern sites, corresponding to relative infestations of Lone Star ticks (Murdock et al., 2009). In a survey of deer in Arkansas, 11% of blood samples from hunter killed deer contained *B.lonestari* DNA (Fryxell et al., 2012). Although it is possible that deer are simply sentinels (exposed to bites of Lone Star ticks), the available evidence is suggestive of their role as an amplification host. *B.lonestari* was detected by PCR in turkeys, suggesting another possible amplification host (Jordan et al., 2009). *B. lonestari* infects host-seeking larval *A. americanum* (Stromdahl et al., 2003), demonstrating that TOT contributes to perpetuation. The stability of *B. lonestari* inheritance, however, has not been measured, and, thus, it is possible that amplifying hosts such as deer or turkeys, both important hosts for maintaining Lone Star tick infestations, are required for perpetuation.

In the early 1990s, BSK cultivated isolates from *I. persulcatus* collected from vegetation in Hokkaido, Japan were determined to differ from *B. burgdorferi* s.l. by DNA-DNA hybridization and by sequencing the 16S rDNA. The newly recognized spirochete, which grouped with the relapsing fever borreliae, was named *B. miyamotoi* in honor of Kenji Miyamoto, who had conducted the tick surveys in Lyme disease endemic sites in Hokkaido, and who had isolated the spirochetes (Fukunaga et al., 1995). This spirochete subsequently was found to infect *I.dammini* ticks used for experimental purposes (Scoles et al., 2001), and the comprehensive studies that followed determined that *P. leucopus* were competent reservoirs. 1.9%-2.5% of host seeking *I.dammini* from Lyme disease endemic sites in New York, Connecticut, Rhode Island and New Jersey were infected and infected female ticks provided filial infection rates of 6%-73%. A study of engorged female ticks removed from deer in 4 different states demonstrated a TOTR of 57%-100%, with FIR of 68%-100%, resulting in a VTR of 44%-93% (Han et al., 2019). Thus, inheritance is likely the main mode of perpetuation if VTR remains stable through additional generations.

Human infection and disease due to *B. miyamotoi* have been reported from Russia, Japan, the northeastern U.S., upper Midwestern U.S., and E. U. countries. *B. miyamotoi* has been detected in host seeking *Ixodes* spp. wherever surveys have been done in Lyme disease endemic sites e.g. (Crowder et al., 2014; Dibernardo et al., 2014; Fomenko et al., 2010; Hamšíková Svitálková et al., 2017; Richter et al., 2003; Wagemakers et al., 2017). Prevalence of infection in host seeking ticks has been determined to be on the order of 1%-2%, although there are reports of greater prevalences, which may represent sampling bias (“luck” of collecting many individuals that may have fed on a single spirochetemic animal or were the progeny of a single infected female).

At least 3 genotypes have been identified, suggesting that, like *B. burgdorferi* s.l., *B. miyamotoi* may comprise a species complex: Asian/Siberian, European, and American, mainly from ticks of the *I.ricinus* complex. *I.* (*Partipalpiger*) *ovatus*, a bird feeding eastern Asian tick, has been reported to be infected by the Asian genotype and what appears to be a fourth genotype of *B. miyamotoi* (Iwabu-Itoh et al., 2017). The Asian genotype of *B. miyamotoi* has also been reported from a single host-seeking *Haemaphysalis concinna* (Jiang, 2018). The detection of *B. miyamotoi* from *H. longicornis* or *H. inermis* removed from hosts (Pere David deer, woodmouse) (Heglasová et al., 2020; Yang, 2018) does not necessarily imply their contribution to perpetuation because these ticks may have acquired infection from the host from which they were removed.

Like *B. burgdorferi* s.l., the reported host range for *B. miyamotoi* is diverse. Deer likely serve as amplification hosts (Han et al., 2016), and the spirochete has been detected from wild boar (Wodecka and Skotarczak, 2016). Surveys of animals demonstrated that rodents (woodmice, deer mice, voles, chipmunk and squirrel (Hamer et al., 2012; Burri et al., 2014; Hamšíková Svitálková et al., 2017; Wagemakers et al., 2017), hedgehog (Jahfari et al., 2017) and raccoon (Barbour et al., 2009) were infected, as were diverse passerine birds (Hamer et al., 2012; Heylen et al., 2017; Wagemakers et al., 2017). A large proportion (60%) of turkeys from Tennessee (Scott et al., 2010) were considered to be infected. The wide range of possible amplification hosts reflects the wide host range of the *I. ricinus* species complex that serve as the main vectors for *B. burgdorferi* s.l. and *B. miyamotoi.* Concurrent infection of the two kinds of spirochetes appears to be rare, on the order of 0.1%-1% and no more than expected by chance (Hamer et al., 2012; Lynn et al., 2018). Indeed, the case has been made that the two spirochetes coexist using one vector by means of niche partitioning (Barbour et al., 2009). In the enzootic cycle with *I. dammini* and *P. leucopus* in New England, *B. burgdorferi* establishes chronic infection of the skin and the pivotal event in the enzootic cycle is transmission by nymphal ticks. *B. miyamotoi* has transient spirochetemia (blood) and transmission is by larvae (Barbour et al., 2009). Even a single *B.miyamotoi*-infected larva is sufficient to infect a host (Breuner et al., 2018).

**Louse borne relapsing fever**

Only two *Borrelia* spp. are anthroponotic, one causing louse borne relapsing fever (also known as epidemic relapsing fever) and one endemic tick-borne relapsing fever in Africa. The former is transmitted by the human louse (*Pediculus humanus*) and derives from endemic (African) tick borne relapsing fever spirochetes (*B. duttoni*) maintained by *O. moubata*-like ticks as an anthroponosis. The human louse has classically been distinguished into head louse (*Pediculus humanus capitis*) and body louse (*P. humanus corporis* or *P. humanus humanus*) based on morphology and feeding behavior. Body lice are not found in the hair but head lice could sometimes be found on the body. The two types were capable of interbreeding and both could be infected by *B. recurrentis* (Buxton, 1946).

As for the other louse-transmitted bacterial infections (trench fever and epidemic typhus), the agent of epidemic relapsing fever (*B.recurrentis*) contains a reduced genome relative to that of its tick maintained parent: 20.4% of the genome has been lost compared with *B.duttoni*, including the genes for the antigenic lipoproteins vlp and vsp as well as DNA repair genes *recA*, *mutS*, and *smf* (Elbir et al., 2014). The two borreliae apparently diverged 6-14 million years ago as determined by 16S rDNA comparison, consistent with the divergence of *P. humanus* from the chimpanzee infesting louse *P. schaeffi* (Reed et al., 2007). This estimate conflicts with the current evidence that body lice have originated multiple times, even within recent history, from head lice (Li et al., 2010), and that new populations may be constantly evolving from head lice. Body lice and head lice are the same species but are different ecotypes (subpopulations that specialize in different niches). It may be that as more *B.recurrentis* isolates are analyzed, the theory of an older divergence will become modified by the findings of more recent generation of local lineages from *B. duttoni.* Indeed, body lice were demonstrated to transmit *B. duttoni* (Heisch and Garnham, 1948), and the spirochete could be passaged indefinitely between lice by enema or intracoelomic inoculation (Mooser and Weyer 1954, as cited in (Geigy, 1968a) with no loss of infectivity to mice.

The perpetuation of *B.recurrentis* depends solely on its human reservoir. The spirochete's distribution, abundance, and relationships with the environment are regulated by those of its louse vector, which, in turn, is regulated by human ecology. Lice become infected only by feeding on people who have active spirochetemia. Apparently, a minimum of 4 spirochetes per microliter (1-2 per oil immersion field; (Heisch et al., 1960) is required to infect a louse. Only about a fifth of lice feeding on infectious people become infected. Within a week of an infectious bloodmeal, spirochetes may be observed in the louse hemolymph. They do not invade the ovarial tissues, hence there is no inherited infection (no transovarial transmission (Nicolle et al., 1912). There is a fitness cost to infection by *B. recurrentis* (and *Rickettsia prowazekii*) in that infected lice tend to die quickly (Houhamdi and Raoult, 2005). Individual lice live 3-7 days (at 30ºC and 10ºC respectively (Nuttall, 1917) in the absence of a bloodmeal and do not enter any dormancy that might prolong spirochetal survival in the absence of a live host. Thus, the louse is not the reservoir, and maintenance depends on humans.

The main mode of transmission is by crushing the infected louse, followed by the penetration of excoriated skin by the liberated spirochetes; *B. recurrentis* does not appear to invade the salivary glands and, thus, is not transmitted by bite. Although classic reviews on the biology of relapsing fever (Felsenfeld, 1971) state that louse feces are not infectious, rabbit adapted human lice infected by feeding on a rabbit that had been intravenously injected with 6 logs of *B. recurrentis* as they fed excreted viable spirochetes (Houhamdi and Raoult, 2005). The duration of infectivity for excreta is not clearly defined, and so, in the absence of extended survival of the spirochetes in dried louse excreta, the general role of fomites in maintaining *B. recurrentis* is likely minor; healthcare workers attending to victims of epidemic typhus often got infected by exposure to louse feces, whereas those attending relapsing fever patients did not (Zinsser, 1935).

Lice can be transferred between people by the social touching of heads (Rózsa and Apari, 2012), although there is some confusion as to whether this pertains to head lice or body lice), sharing clothing or bedding, by migration of lice from “verminous people” (Nuttall, 1917), or perhaps by deliberate infestation. Malabar holy men once apparently voluntarily let themselves be infested; young women in northern Siberia threw lice at visitors (Zinsser, 1935).Other than interesting cultural practices, prolonged close contact is required to maintain lice, such as under conditions of war, migration or during winter. Louse-borne relapsing fever is less common and more geographically restricted due to improved hygiene. Once upon a time, louse borne relapsing fever was likely cosmopolitan in distribution, traveling with people on ships and on war marches as did epidemic typhus (Zinsser, 1935). The Horn of Africa continues to remain endemic for this infection(de Jong et al., 1995; Nordmann et al., 2018; Yimer et al., 2014), with sporadic outbreaks. Large outbreaks may continue to occur where there are large accumulations of displaced people and a breakdown in hygiene and healthcare. In Sudan refugee camps, a 10% mortality was estimated to have occurred in 1999-2000 (Raoult and Roux, 1999). No country is immune from potential outbreaks: the EU has reported numerous louse borne relapsing fever cases in immigrants from the Horn of Africa, with spillover infections in people from other countries sharing the same migrant camps (ECDC(2015).

**Comments on how to advance our knowledge of the “ecology of borreliae”.**

An ecologist will use an intervention to probe hypotheses about perpetuation; an epidemiologist will seek to discover the main elements of perpetuation as it relates to risk to either explain the public health burden (distribution and abundance of human infections), or formulate interventions, with the expectation that the results will reduce risk. Much of the past and current “Lyme disease ecology” literature is based on observational studies driven by epidemiology. We anticipate that the next version of this chapter will have a greater number of experimental ecology reports from which to draw. The new tools that are available will facilitate perturbation of nature. For example, Mice Against Ticks (MAT), a project that seeks to replace reservoir competent *P. leucopus* in zoonotic sites with those that are genetically modified to be less infectious to ticks (Buchthal et al., 2019), will provide the means to analyze whether there are essential species for enzootic *B. burgdorferi* transmission, as well as reduce BRN overflow. Other possibilities include the introduction into sites of ticks containing borreliae with genetic markers (e.g., GFP), although (as with MAT) there would be substantive ethical and regulatory issues that would require resolution.

One of the most vexing aspects of quantifying enzootic transmission (and entomological risk for zoonotic transmission) of *B. burgdorferi* s.l., as well as other hard tick transmitted borreliae, is the reliance on a single index: the entomological inoculation rate (EIR), or density of infected nymphs. This is the product of the prevalence of infection and the density of nymphs; much energy and resources are expended in the measurement of these indices without much thought into what they mean. EIR has its origins in malarial epidemiology and with caveats has some uses in measuring zoonotic risk of tick borne infections, mainly at larger scales and not at that of yards or small study sites (Eisen and Eisen, 2016). However, the utility of EIR for analyzing enzootic transmission is not axiomatic. Measuring the density of the tick vectors for *B. burgdorferi* is not straightforward. Although dragging (flagging, sweeping) to collect host seeking ticks provides an index of abundance, such an index is prone to error because the availability of ticks to be sampled depends on the abundance of hosts (Ginsberg and Ewing, 1989), and is affected by temperature and humidity at the time of sampling, accessibility of suitable habitat, and even inter-investigator prowess.

Indeed, sites are often selected because they yield many ticks as opposed to others (sample size is critical for measuring prevalence of infection), and are easier to traverse. Many sites would not be representative of what a mouse, shrew or bird would encounter; such animals go where we cannot. Standardizing transect selection, conditions and methods can reduce the variability that otherwise plagues measuring tick density, but drag sampling is a human risk-biased snapshot of the venue of enzootic transmission. Then too, all ticks from the transect (typically 500m or so, with a minimum of 20-30 minutes, usually 60 minutes of sampling) are placed into a single collection vial, ignoring the clustering of ticks and even infected ticks (Foppa et al., 2002; Telford et al., 1992); most 20m drags yield 0 or 1 ticks, and a small number >3, and an even smaller number may have dozens per drag. The clusters themselves provide a clue to the venue of transmission: subadult ticks engorge and drop from their hosts at the start of the host's activity (Mather and Spielman, 1986) and may accumulate in a mouse burrow, for example. By ignoring the clusters and averaging across a transect, we may be missing even more of the complexity of enzootic transmission, as we found in a study of focality for *Francisella tularensis* infection of *Dermacentor variabilis*(Goethert and Telford, 2009); bacterial diversity appeared to originate from a 260m diameter focus along a 1500m transect. We need to find better and complementary ways of estimating and interpreting infected and noninfected host seeking tick abundance, particularly where animals are most commonly encountering ticks.

Infestation of animals provides less biased indices of abundance for ixodid ticks, although the indices depend on number of animals examined as well as investigator experience in counting ticks on animals. Capture-mark-release is the standard method for censusing animal populations but to date has not been effectively used for *Ixodes* ticks (methods of marking that minimize fitness effects but are durable are expensive, e.g., the use of rare metals such as rubidium ingested in a bloodmeal, and would only provide estimates for host-seeking ticks but not those that are in the process of molting (which are difficult to collect). Microcosm studies (e.g., (Arsnoe et al., 2019) can provide good data on survival and questing behavior, but it may be logistically difficult to use microcosms to study the abundance of ticks or transmission of borreliae. The force of enzootic spirochetal transmission may be estimated by seroprevalence (or better, seroconversion), or direct detection of the agent by culture or PCR of animal skin samples. Xenodiagnosis is the best of all, but extremely labor intensive. Of all the current methods for analyzing BRN, measuring the infection status of enzootic hosts is the least biased if performed longitudinally. More objective means of sampling host seeking ticks would provide the complementary aspects of modeling BRN.

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