Fundamental processes in the evolutionary ecology of Lyme borreliosis

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Abstract | The evolutionary ecology of many emerging infectious diseases, particularly vector-borne zoonoses, is poorly understood. Here, we aim to develop a biological, process-based framework for vector-borne zoonoses, using *Borrelia burgdorferi* sensu lato (s.l.), the causative agent of Lyme borreliosis in humans, as an example. We explore the fundamental biological processes that operate in this zoonosis and put forward hypotheses on how extrinsic cues and intrinsic dynamics shape *B. burgdorferi* s.l. populations. Additionally, we highlight possible epidemiological parallels between *B. burgdorferi* s.l. and other vector-borne zoonotic pathogens, including West Nile virus.

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Evolutionary ecology is an amalgamation of evolutionary biology and ecology, which considers evolutionary relationships and genetic changes in species or populations as well as their interactions with each other and with the environment. Two questions are central to the scientific debate in evolutionary ecology: how do population fluctuations arise and how is diversity generated and maintained[1,2]? Despite different terminology, the same fundamental questions are at the centre of contemporary infectious disease epidemiology[3].

The prevailing trend towards the development of unifying frameworks for understanding infectious disease dynamics recently culminated in the development of a concept known as 'phylodynamics', a framework to describe how the genetic variation of a pathogen is influenced by epidemiology, the dynamics of host immunity, host and pathogen population survival, and the phylogeny of the pathogen[4]. For a few pathogens (for example, HIV, influenza virus, dengue virus and measles virus), the work leading to the development of this framework has resolved several crucial issues, including the mechanisms involved in the evolution of diversity[4,5].

Gog and Grenfell coined the term 'strain space' for directly transmitted pathogens, and the geometry of this space is described by two processes: host immunity and the rate of genetic change of the pathogen[6]. Many emerging infectious diseases are transmitted by vectors, mainly ticks and insects[7], and for these diseases, vector-related processes could further define the strain space[8,9]. Therefore, it is essential to understand vector-related processes to decide to what extent the principles that are common to directly transmitted pathogens can be extrapolated to vector-borne pathogens[10].

Current models of vector-borne zoonoses are theoretical, except for models that aim to capture the spatial–temporal distribution of these zoonoses using statistical, pattern-matching approaches[9–11]. Although statistical models can shed light on biological processes if used correctly, models based on biological processes are thought to be more powerful in illuminating the underlying key biological mechanisms that generate the observed spatial–temporal distribution patterns[10]. Such models should be able to identify geographical and climatic variables a priori that can be used to predict pathogen and disease occurrence in both time and space. The scientific community has now realized that the development and parameterization of biological, process-based models of vector-borne diseases is a timely, feasible and important task that is necessary to predict, prevent and control disease[4,10,12].

Mathematical modelling of multi-strain, multi-host pathogens remains a major challenge[12,13]. As shown recently for directly transmitted pathogens, however, comparing the transmission dynamics of different systems can be a powerful means to explore the roles of different biological processes in shaping pathogen populations[3]. Here, we aim to set out the framework needed to develop biological, process-based models of vector-borne zoonoses, using *Borrelia burgdorferi* sensu lato (s.l.), the spirochaete agent of Lyme borreliosis, as an example. By

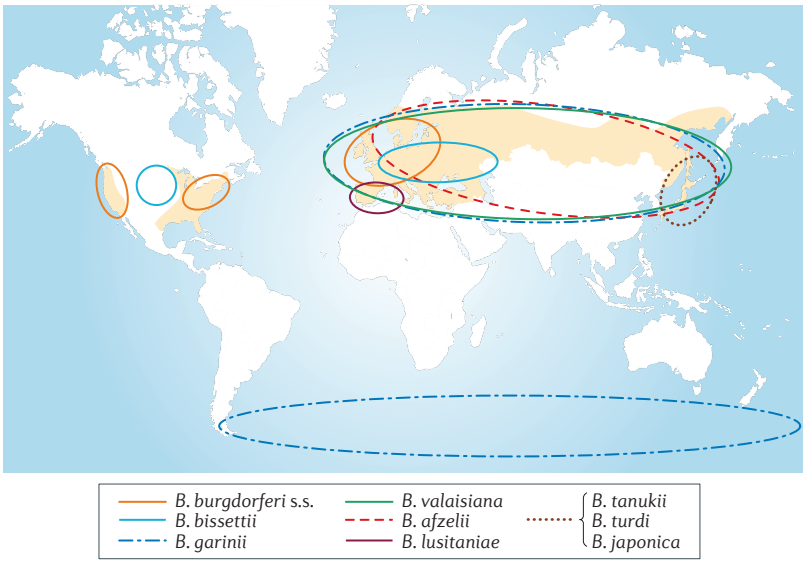


Figure 1 | **The geographical distribution of *Borrelia burgdorferi* sensu lato.** The beige-shaded background indicates the geographical distribution of recorded clinical cases of Lyme borreliosis[38]. The highest species richness is recorded for Eurasia. In the northeastern United States, *Borrelia burgdorferi* sensu stricto (s.s.) is expanding in population size and geographical range, causing epidemics of Lyme disease in humans. *Borrelia afzelii* seems to be much less abundant in the British Isles compared with continental Eurasia. The prevalence of *B. burgdorferi* s.s. phases out towards eastern Europe. *Borrelia garinii* and *Borrelia valaisiana* are found across much of terrestrial Eurasia. *B. garinii* is also maintained by seabird species and *Ixodes uriae* ticks in pelagic transmission cycles in both hemispheres. *Borrelia bissettii* and *Borrelia lusitaniae* have occasionally been found in locations beyond their core range. *Borrelia andersonii* in the United States, *Borrelia sinica* in Asia and *Borrelia spielmanii* sp. nov. in Europe are omitted from the figure, because there is little information on their distribution.

comparing different natural transmission cycles (that is, how the bacterium is maintained in different vector and host systems) and by reviewing advances in our understanding of transmission at the vector–host interface, we aim to identify the key processes that drive the evolutionary ecology of *B. burgdorferi* s.l., which must then be accounted for in mathematical models. We put forward hypotheses on how the spirochaete populations respond to these processes, which comprise extrinsic cues and intrinsic dynamics, and discuss the impact of the host community on this zoonosis through the dilution effect[14,15] and multiple niche polymorphism[16,17]. Last, we highlight possible ecological parallels and differences among the different members of the *B. burgdorferi* s.l. species complex and other important vector-borne pathogens, suggesting that there could be common principles in their evolution.

The basic biology of Lyme borreliosis

Lyme borreliosis was first identified almost three decades ago in Old Lyme, Connecticut, USA[18], and the causative agent was shown to be a tick-borne spirochaete[19], later named *Borrelia burgdorferi*. Genetic analyses of spirochaete strains from other parts of the world have revealed that the spirochaetes associated with Lyme borreliosis form a species complex known as *B. burgdorferi* s.l., which comprises 12 named species, including *B. burgdorferi* sensu stricto (s.s.), *Borrelia garinii* and *Borrelia afzelii*, which are pathogenic in humans[20–22] (FIG. 1). In temperate zones, Lyme borreliosis is the most frequent vector-borne disease of humans.

The complex zoonotic transmission networks of *B. burgdorferi* s.l. are maintained in nature by ixodid (hard) tick species and a broad spectrum of transmission-competent vertebrate hosts (the so-called reservoir hosts that infect ticks), such as rodents, insectivores and several bird species[23,24]. A hallmark of the ecology of these spirochaetes is the wide variation in the breadth and pattern of host specialism[24–28]. *B. burgdorferi* s.l. can therefore be regarded as a group of different ecological populations, or ecotypes[10,29]. It is important to note that *Ixodes scapularis*, *Ixodes ricinus* and *Ixodes persulcatus*, the principal tick vectors in the northeastern United States, Europe and Asia, respectively, are all generalist ectoparasites; that is, they feed on many different vertebrate species. The generalist nature of these vectors provides opportunities for transmission between host species, potentially linking the different ecological niches of the spirochaetes[10,30–32].

Ixodid ticks differ from insects[33]. Unlike insects, each of the developmental stages of an ixodid tick (larva, nymph and adult female) feeds once on a vertebrate host for several consecutive days, followed by prolonged interstadial development. Extrinsic abiotic factors, such as temperature, affect the host-seeking behaviour and phenology of ticks (BOX 1). For efficient infective cycles of *B. burgdorferi* s.l. to thrive over time, tick larvae must acquire the bacteria from infectious hosts and subsequently transmit them to susceptible host populations as nymphs; the role for adult ticks as vectors is limited in most cases, because adult males do not feed and in most habitats adult female ticks feed mainly on large animals, such as deer, that are not competent reservoir hosts[23,30–34]. The extrinsic incubation period of spirochaetes in ixodid ticks equals the duration of development from the larval to nymph stages, which is climate sensitive[9,33]. This period can last for up to a year for hard ticks in temperate zones[34] (BOX 1). Spirochaete populations are therefore shaped substantially by the environmental cues that operate on the tick populations. Additional layers of complexity are introduced to the system by host population dynamics and the host immune response to both the bacteria and the ticks (see below)[23,35–37]. Theoretical studies indicate that these complexities will probably yield wide spatial–temporal fluctuations in the relative abundance of different spirochaete genotypes[1–3,6,12].

Ecological maintenance patterns

The ecological maintenance systems and intrinsic transmission dynamics of *B. burgdorferi* s.l. are diverse. Here, we describe selected regional examples that represent the global range of ecological and epidemiological variation (TABLE 1).

Northeastern United States. Lyme borreliosis in this region is caused by one species, *B. burgdorferi* s.s. (FIG. 1). Since the identification of Lyme borreliosis the incidence of clinical cases has increased, with an apparent peak in 2002 (REF. 38). In addition to human encroachment

Vectors
Organisms that transmit microorganisms between hosts, either through mechanical or biological transmission. Many disease vectors are invertebrates, particularly insects and ticks.

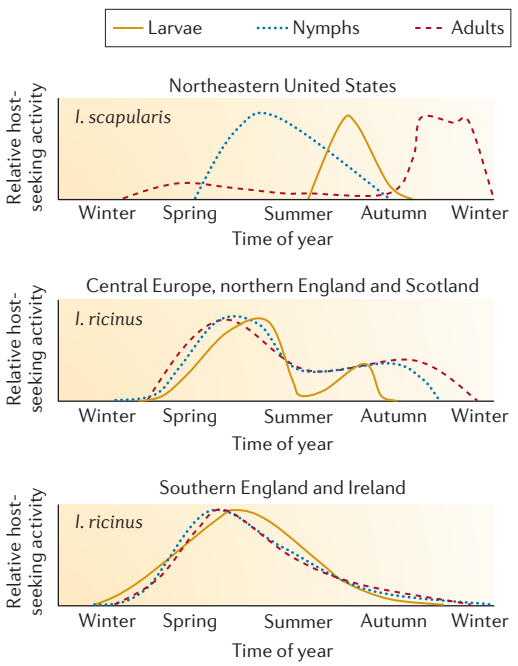
Vector-borne zoonoses
Diseases caused by pathogens that are maintained by animal populations and which can infect the human population through vectors.

Dilution effect
According to this concept, the transmission intensity of vector-borne zoonotic pathogens is reduced (that is, diluted) in species-rich host communities because many host species are inefficient in infecting vectors.

Box 1 | R_0 and the host-seeking phenology of ticks

The basic reproduction number, R_0, must be >1 for pathogens to survive in host populations[64]. Many variables in the equation below, which was developed for tick-borne pathogens[33], are affected by biotic factors and extrinsic, abiotic factors (in particular, temperature and relative humidity) that affect the tick and host populations[33,90–93,98]. The extrinsic incubation period, *n*, is a particularly important parameter because it is a power variable. A shorter extrinsic incubation period and increased tick abundance, both of which are sensitive to climate[90], positively affect R_0. The transmission coefficient, β, and the daily rate of loss of infectivity, *r*, in hosts vary with *Borrelia burgdorferi* sensu lato (s.l.) strain and host population. Cross immunity among strains, for example, would reduce β and, at the same time, increase *r* for a specific spirochaete strain, thereby reducing R_0 (FIG. 2).

As shown in the figure, the different developmental stages of *Ixodes scapularis* in the northeastern United States are active at different times of the year. Asynchrony of nymphs and larvae seems to select for long-lived spirochaete strains. In Europe, all stages of *Ixodes ricinus* are active for a longer period than the developmental stages of *I. scapularis* in North America, a feature that also favours persistent strains of *B. burgdorferi* s.l., despite synchronous activity patterns of the tick stages. In Ireland and, perhaps much of England, nymphs, the principal vector stage of *I. ricinus*, seem to display different host-seeking behaviour. Experimental work and ecological field studies indicate that they might quest higher in the herbage than their continental counterparts[47,92]. This could explain why small rodents are rarely infested with nymphs of *I. ricinus* in the British Isles, and also why *Borrelia afzelii*, a rodent-associated species, seems to be relatively rare. These examples illustrate how climatic forces can shape the population structures of pathogens by affecting their vectors. The *y*-axis in the figure shows the relative host-seeking activity for each developmental stage on an arbitrary scale and does not give information about the absolute abundance of the ticks.



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

N/*H*, ratio of vectors to hosts; *f*, probability of tick feeding on a host species; $\beta_{V-T}\,\beta_{T-T}\,\beta_{T-V}$, product of the three transmission coefficients; *p*, vector's daily survival probability; *n*, extrinsic incubation period; p^n, interstadial survival rate; *F*, vector's reproduction rate; *r*, daily rate of loss of infectivity in the host; *h*, host's daily mortality rate. All variables, except *F* and *r*, are known to be affected by climate.

Multiple niche polymorphism
A form of frequency-dependent polymorphism in which different genotypes of a species display different fitness in different niches. In the case of pathogens, different niches can correspond to different host populations or species. A stable polymorphism can evolve more easily if there is habitat selection.

Host specialism
Generalist pathogens use a range of phylogenetically distant hosts, whereas specialist pathogens infect or infest only one or a few more closely related host species. Even very closely related pathogens can display substantially different levels of host specialism.

Ecotypes
An ecotype comprises organisms that share the same ecological niche.

Phenology
The seasonal cycle of development and activity of the different developmental stages of ticks.

Epizootic spread
The animal equivalent of epidemic spread of pathogens among humans.

into tick habitats, this rise has a substantial biological component; that is, there has been epizootic spread of the bacterial populations[8,30,34,39,40]. Two factors have facilitated the spread of the bacteria in this region; first, the increasing population size and geographical range of *I. scapularis* ticks, which is believed to be driven by restored woodlands and growing populations of deer, an important reproductive host of ticks[34,39–41]; and second, the ability of *B. burgdorferi* s.s. as a generalist to infect a wide range of phylogenetically distant vertebrate hosts, including mammalian and avian species[25,28]. It is likely that any further expansion of the range of *I. scapularis* ticks, and therefore of Lyme borreliosis, will ultimately be restricted by climate and perhaps also by photoperiod[30]. This is supported by the fact that the current range of white-tailed deer is much larger than that of *I. scapularis*, covering much of North America and parts of central America.

Continental Europe. At least three of the seven recorded species of *B. burgdorferi* s.l. found in continental Europe cause disease in humans, of which *B. afzelii* and *B. garinii* are the most abundant species[23,24,32] (FIG. 1). The broad temporal pattern of the incidence of Lyme borreliosis in Europe seems to be more stable than in the northeastern United States, possibly owing to the reasons discussed in the following section, although local temporal fluctuations in the infection prevalence of the bacteria in ticks have been recorded[42]. Another recent study has detected a high focal prevalence of *B. garinii* outer surface protein A (OspA) serotype 4, a recently evolved, hyperinvasive and aggressive genotype[43]. It is therefore possible that we are currently witnessing the onset of an epizootic spread of this novel genotype across Eurasia.

Unlike in the northeastern United States, in Europe, most species or even subtypes of *B. burgdorferi* s.l. are specialized to infect different groups of vertebrates[23,24]. For example, OspA serotype 2 of *B. afzelii* and OspA serotype 4 of *B. garinii* are associated with rodents and some insectivore species[26,32,44], whereas *Borrelia valaisiana* and OspA serotypes 3 and 5–8 of *B. garinii* are maintained by terrestrial birds and seabird species[24,27,32]. The specialism of the European *B. burgdorferi* s.l. strains limits transmission between different host species, which probably stabilizes the abundance patterns of the different *B. burgdorferi* s.l. strains.

Table 1 | **Ecological maintenance patterns of Lyme borreliosis in different regions of the world**

Region	Temporal stability	*B. burgdorferi* s.l. species richness	Tick vector	Infection prevalence in questing nymphs	Reservoir community of *B. burgdorferi* s.l.	Host-seeking phenology of ticks
Northeastern USA	Epizootic/ epidemic	1	*Ixodes scapularis*	25–35%	Several mammalian and avian species	Asynchronous
Continental Europe	Enzootic/ endemic	7	*Ixodes ricinus*	~10%	Several mammalian and avian species	Synchronous and unimodal in western parts and bimodal in central parts
British Isles	Enzootic/ endemic	4	*Ixodes ricinus*	2–10%	Several avian species, squirrels	Synchronous and both unimodal and bimodal
Pelagic systems	Enzootic/ cryptic	2	*Ixodes uriae*	2–10%	Seabirds	Synchronous

B. burgdorferi s.l., *Borrelia burgdorferi* sensu lato.

British Isles. The dominant species here are *B. garinii* and *B. valaisiana*, and there are no published records on the abundance of *B. afzelii*, a rodent-associated species that is one of the most prevalent in continental Europe[45,46] (FIG. 1). In a recent field study from south-west England, however, *B. afzelii* was found in questing ticks, but at a low prevalence (K.K. and R. Mitchell, unpublished observations). Pheasants and some seabirds are among the most important avian reservoir host species that maintain *B. garinii* and *B. valaisiana*[45]. Although small rodents are important components of the reservoir host community in most Lyme borreliosis maintenance systems worldwide, these mammals seem to have much less important roles as reservoirs in most of the British Isles[45,46]. The most likely reason for the almost complete 'loss' of this functional host group is that nymphs (the main vector stage) of *I. ricinus* rarely feed on small rodents in this region (this phenomenon is most pronounced in Ireland)[47]. It has been suggested that this behavioural feature of the tick is driven by climate[47], which might explain the low prevalence of *B. afzelii* (BOX 1). However, more detailed and geographically widespread studies are needed to investigate nymphal infestation of small rodents and the prevalence of *B. afzelii* infection in the British Isles before definitive conclusions can be drawn.

In an additional subsystem in the British Isles, *B. burgdorferi* s.s. is maintained by sheep through co-feeding of nymphs and adults[48].

Fundamental biological processes

In this section, we explore biological processes that are involved in the maintenance of natural transmission cycles and gene flow in *B. burgdorferi* s.l. populations, and identify those that might be key to the evolutionary ecology of these spirochaetes.

Selection in the host. Host association (host specialism) of pathogens implies that there are barriers to cross-species transmission. The host specialism of *B. burgdorferi* s.l. is a result of negative selection that is mediated by the alternative pathway of the complement system[23,24,49]. Complement-resistant spirochaetes deflect this arm of innate host immunity by binding to host-derived complement inhibitors through their outer-surface Erp proteins and a few other plasmid-encoded proteins[50–52]. By contrast, spirochaetes that are sensitive to destruction by the complement system of a particular host species are lysed early in the midgut of the feeding tick, and are thereby eliminated by the host[53]. These findings have led to the hypothesis that the host range of a spirochaete strain is restricted by its repertoire of genes that encode ligands with high binding affinities for complement inhibitors[24,51]. Therefore, *B. burgdorferi* s.l. is one of the few zoonotic pathogens for which molecular mechanisms for host specialism or generalism have been proposed[54].

Adaptive immunity is another important player in the biology of *B. burgdorferi* s.l.[23,35,36,55], because it introduces frequency-dependent (that is, non-linear) processes. It has been suggested that balancing selection that acts on the *ospC* gene or linked loci is a dominant force maintaining spirochaete diversity within local tick populations[8,17]. Theoretical studies predict that frequency-dependent fitness leads to fluctuations in the abundance of spirochaete genotypes, which would result in temporal shifts in the population structures[1–3]. Temporal shifts in the frequency distribution of genotypes have indeed been shown for *B. burgdorferi* s.s. populations in questing ticks from the northeastern United States[8]. Strain interferences mediated by cross immunity can further complicate the population biology of *B. burgdorferi* s.l.[6,56] (FIG. 2). Time-series analyses are required to assess the scale of spatial–temporal variation in the abundance of spirochaete genotypes[2].

Evolutionary theory predicts that selection favours specialism of pathogens if hosts are abundant, whereas generalist strategies should evolve under conditions in

Complement
An important component of the innate immune response of vertebrate hosts. Its main function is first-line defence against invading microorganisms. Most extracellular pathogens of vertebrate hosts have evolved mechanisms to resist complement-mediated clearance by the host.

Frequency-dependent fitness
Fitness describes the capability of an organism to reproduce. Frequency-dependency fitness is an important characteristic of pathogens, because host herd immunity reduces the fitness of pathogens as they increase in prevalence, thereby regulating the population size of the pathogen.

Strain interference
Strain interference among pathogens occurs if the presence of one strain affects the fitness of a co-infecting strain, either through direct competition or indirectly through cross immunity.

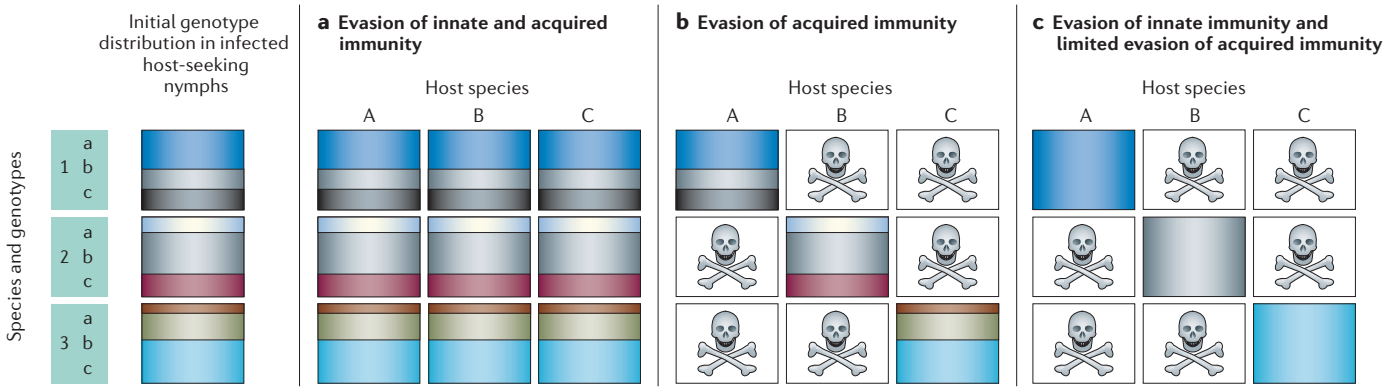


Figure 2 | **Different scenarios of interference among *Borrelia burgdorferi* sensu lato (s.l.) species and genotypes and immune evasion.** The initial relative prevalence of different *B. burgdorferi* s.l. species (1, 2 and 3) and genotypes within species (a, b and c) in questing nymphs is indicated by the relative size of the different coloured boxes on the left-hand side. The theoretical community comprises three host species for *B. burgdorferi* s.l. and ticks (A, B and C), which have the same abundance at all times and mortality rates that are unaffected by infection. The relative prevalence of different species and genotypes in larvae that are infected by host species is indicated by the size of the boxes under each host species for each of the scenarios. In scenario **a**, all genotypes of all species are equally able to evade the innate and acquired immune responses of all host species. Differences among genotypes in the prevalence of infection in larvae that feed on the hosts are innate characteristics of the bacteria and are not immune-mediated. In scenario **b**, all genotypes of a species are equally able to evade the acquired immune response of a host species, but can only evade the innate immune response of one host species, which corresponds to our current understanding of the genetic diversity and ecology of *B. burgdorferi* s.l. in Europe[24,49]. In scenario **c**, the pattern of susceptibility of *B. burgdorferi* s.l. genotypes and species to host innate immunity is the same as in scenario **b**, but the bacteria are successful in evading host acquired immunity for a short period only. As cross immunity to the most similar genotypes develops, hosts are most often infected first with the fittest and most abundant genotypes, and less abundant genotypes die out (indicated by skull and cross bones).

which encounters with host species are less predictable[54,57–60]. Therefore, it is tempting to speculate that the generalist strategy of *B. burgdorferi* s.s. in the north-eastern United States echoes adaptation to large-scale habitat destruction and near-extinction of deer during the post-Columbus settlement period[28,39]. The relatively greater degree of specialism of most strains of *B. burgdorferi* s.l. in Eurasia reflects a different natural history and demography of these bacteria, perhaps because vast sylvatic refuges have remained relatively undisturbed since the last Ice Age.

One of the most important parameters in the biology of infectious agents is the timescale of infectivity relative to the lifespan of the host[6]. A long-standing paradigm in Lyme borreliosis research was that infections in susceptible hosts persist for the lifetime of the host in the presence of strong strain-specific immunity[36,61]. However, it has been shown for certain spirochaete strains that the infectivity of rodents for ticks declines within a few weeks after infection; therefore strains vary in the duration of infection in rodents, and potentially in other reservoir host species[56,62].

Such differences have stark consequences for the dynamics of natural transmission cycles and for the choice of the structure of the mathematical models used to investigate them (FIG. 3). Susceptible-infected (SI) models are appropriate for pathogens that cause long-lived infections in hosts and that remain infective for ticks at a constant level, as typified by the classic *B. burgdorferi* s.s. infections in white-footed mice[61,63]. Susceptible-infected-resistant (SIR) models describe the ecology of pathogens that are short-lived in the host owing to an effective and persistent immune response, that is, a 'boom-and-bust' strategy[56,64], whereas susceptible-infected-susceptible (SIS) models describe the ecology of pathogens that are short-lived in the host but the hosts remain susceptible to reinfection[65,66]. Even for pathogens that are highly infective for ticks only a short time, hosts often do not recover fully and remain persistent carriers[67]. In fact, most tick-borne pathogens have an initial 'boom' acute phase that does not quite 'bust' owing to partial evasion of the host immune response, that is, hosts become carriers that persistently transmit infection with low efficiency. Examples include *Theileria parva*, *Babesia* spp., *Anaplasma marginale* and *Anaplasma phagocytophilum*, as well as some strains of *B. burgdorferi* s.s.[56,67–71]. Models that describe these types of host–pathogen dynamics are more appropriately termed susceptible-infected-carrier (SIC) models[68]. In Europe, efficient transmission of some tick-borne pathogens, including *B. burgdorferi* s.s. and *B. afzelii*, between co-feeding ticks[48,72] is superimposed on transmission from systemically infected hosts and could perhaps be accounted for as an SIS-type component to SIR or SIC models. These transmission characteristics, which arise from the effectiveness of immune evasion and the kinetics of pathogen dissemination, substantially shape the ecology of most pathogens[64].

Migration. As selection and migration of pathogens can generate similar population structures, inferring migration rates from population structures can be fallacious. However, information on host specialism allows

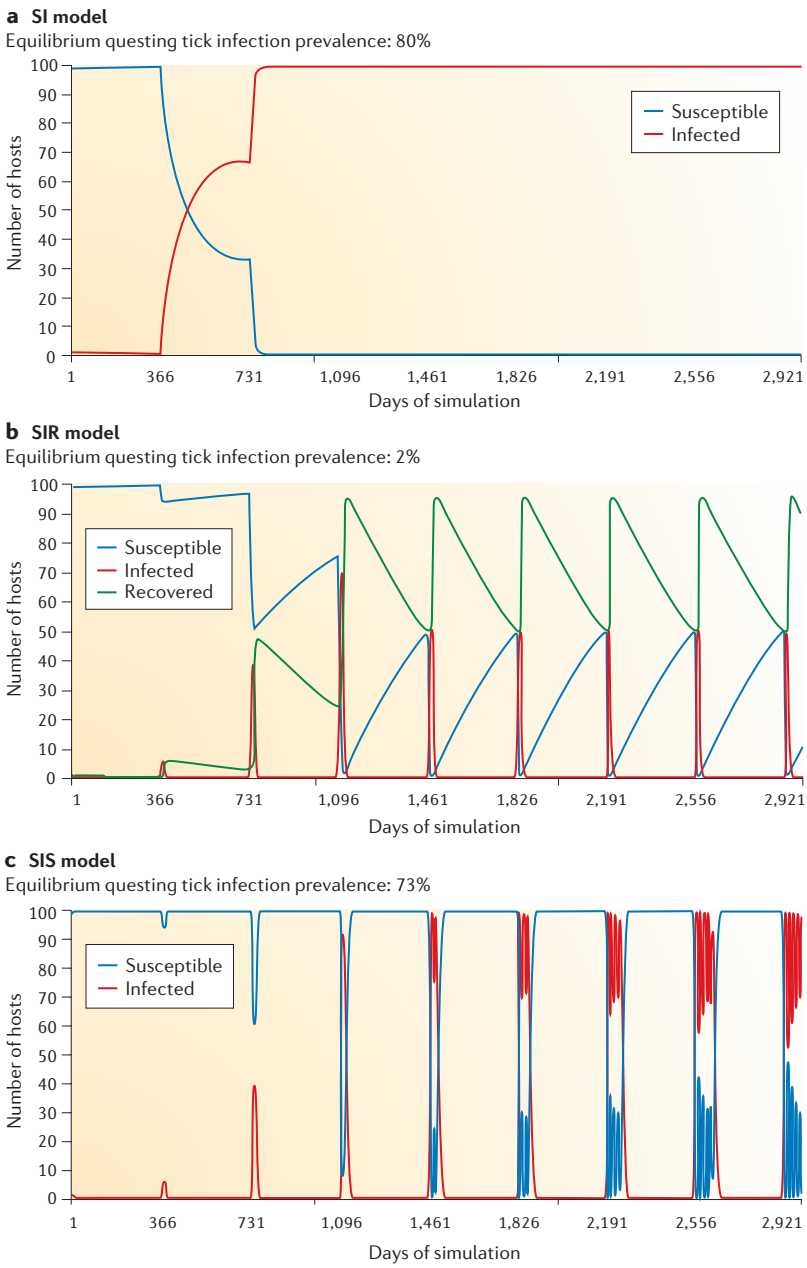


Figure 3 | **Deterministic models of host–pathogen interactions.** Susceptible-infected (SI), susceptible-infected-resistant (SIR) and susceptible-infected-susceptible (SIS) models are compared using three simple theoretical models. Model 1, the SI model, illustrates the classic lifelong *Borrelia burgdorferi* s.s. infection in *Peromyscus leucopus* mice[61]. Almost all hosts are infected by the second year, and newborn hosts rapidly acquire infection leading to a high infection prevalence in hosts and ticks. In model 2, the SIR model, with annual 'boom-and-bust' cycles of infection, there is complete recovery from infection and birth of susceptible hosts, resulting in a low prevalence of infection in hosts and ticks. Model 3, the SIS model, illustrates the potential effect of efficient co-feeding transmission, in which infected hosts become susceptible again, maintaining an overall high prevalence of infective hosts whenever infected ticks are present. This results in a high overall infection prevalence in questing ticks. These models illustrate how variations in host infection and transmission dynamics can result in different epidemiological behaviour and pathogen abundance to which the complexity of seasonal phenologies of vector and host population processes is added in nature. The models were generated in STELLA 7.0.3 for Windows software; High Performance Systems Inc. New Hampshire. Full details of the model parameters can be found in Supplementary Information S1 (box).

the development of process-based hypotheses about the spatial dynamics of pathogens. West Nile virus (WNV) is an example illustrating how the use of highly mobile avian hosts allows a pathogen to disperse across an entire continent within a short period[73]. Most *B. garinii* strains in Europe are associated with birds, and it is therefore intuitive to expect that the migration rates of such strains are high, as has been observed[23,74] (K.K. and S.M. Schaefer, unpublished observations). We propose that the relative uniformity of the frequency distribution of the generalist *B. burgdorferi* s.s. genotypes in the north-eastern United States is partly generated by the migration of infected birds and medium-sized mammals, such as raccoons[28], rather than by balancing selection alone[8].

Mutation and recombination. Several loci of *B. burgdorferi* s.l. seem to be part of an ecologically or immunologically bound linkage group, which has given rise to the popular model of clonal evolution of these organisms[75,76]. This dogma has recently been challenged[77]. The *erp* genes, for example, represent prophage genomes for which transduction has been shown to be a mechanism of reshuffling genetic material among spirochaete strains[78]. As these genes seem to determine the spirochaete ecotype[23,24,29,51], the horizontal transfer of prophage DNA among spirochaete strains might hold the key to the adaptive radiation of *B. burgdorferi* s.l. Ecological processes, particularly the dynamics of infection in host and vector populations, will determine the opportunity for mixing of different genotypes in ways that allow the horizontal transfer of genetic material. These processes will then be key to the rates and trajectories of genetic change in *B. burgdorferi* s.l. populations.

Vector–pathogen relationships. The involvement of a vector can introduce further dimensions to the strain space as defined for directly transmitted pathogens[6]. First, the transmission of a pathogen by a vector, such as a tick, might confer advantages over direct transmission because, among other reasons, arthropod saliva is often immunosuppressive[79]. Some natural hosts, however, acquire resistance to ticks, a process that can contribute to vector regulation and interfere with pathogen transmission[37]. Second, to be transmitted between hosts the pathogen must survive in the different physiological environment of the tick, which has its own immune system that must be evaded[80]. For *B. burgdorferi* s.l., the tick moulting process seems to be particularly hazardous[81], creating population bottlenecks that probably favour genetic drift. Third, several specific molecular interactions between spirochaetes and ticks are required for transmission[82,83]. However, the expression of bacterial proteins that are essential for transmission by the tick can be costly elsewhere in the life cycle, as exemplified by the OspC protein of *B. burgdorferi* s.l.: on the one hand, OspC is necessary to establish early infection in the host, and perhaps also to invade the tick's salivary glands[84,85] but, on the other hand, OspC induces a potentially detrimental immune response in the vertebrate host[36,55]. The outcome of this apparent evolutionary 'arms race' between bacteria and host is an interesting strategy for

immune evasion: spirochaetes downregulate OspC during chronic infection in the host[86,87]. Last, the tick could be a genetic 'mixing vessel' for spirochaetes, because, unlike in the host, the spirochaete load in questing ticks can reach high levels[88]. Because this can increase the rates of genetic recombination among the bacteria, the tick vector itself might shape the evolution of the bacteria.

Ecological theories and Lyme borreliosis

We have identified processes in the biology of *B. burgdorferi* s.l. that are crucial for the survival of the spirochaete in natural transmission cycles and for determining the rates and routes of exchange of genetic material. However, the dynamics of tick-borne bacterial infection in the host can operate on a similar timescale as that of the phenology of seasonal vector and host population processes. All these processes must be considered together in mathematical models that aim to understand how they affect the basic reproductive number, R_0, and therefore the fitness, of tick-borne bacteria (BOX 1). How do they interact to influence the evolutionary ecology of these bacteria?

What selection pressures shift the balance in favour of either 'boom-and-bust' or persistent carrier strategies, or local versus systemic dissemination of the spirochaetes in the host? Maintenance of *B. burgdorferi* s.l. depends on more than one developmental stage of the tick feeding on individual hosts, which in turn is influenced by abiotic factors, particularly relative humidity, which affect the height at which different stages quest in the herbage[47]. Photoperiod and temperature determine tick development rates, thereby influencing the seasonality of different tick instars[30,32,34,89–93] (BOX 1). Randolph and colleagues have already shown that synchrony of infecting nymphs and uninfected larvae of *I. ricinus* in time, space and host population is crucial for the maintenance of western tick-borne encephalitis (WTBE) virus, which causes only a short-lived viraemia in mice[92].

We propose that the phenology of ticks shapes the evolution of transmission characteristics of tick-borne pathogens (BOX 1). Specifically, we predict that asynchrony of infecting nymphs and uninfected larvae favours pathogen persistence strategies, whereas synchrony of these tick stages combined with a short annual period of activity should favour short-lived 'boom-and-bust' strategies and the capacity for co-feeding transmission. In the northeastern United States, where the tick phenology is asynchronous owing to abiotic factors[89,90], and white-footed mice predominate as hosts, most *B. burgdorferi* s.s. genotypes detected are genetically related to strains that have been shown experimentally to persist in white-footed mice, whereas strains of *B. burgdorferi* s.s. that show 'boom-and-bust' behaviour in white-footed mice are rare[17,28,30,56,61,94]. Because of the more moderate climatic conditions in western Europe, the duration of host-seeking activity of all tick developmental stages throughout the year is much longer than in the northeastern United States[32]. Therefore, with the exception of high latitude areas, where the annual period of tick activity is short, long-lived *B. burgdorferi* s.l. infections, which give rise to many more infected ticks than short-lived infections, will be selected for, irrespective of whether or not the tick stages are synchronous. Persistence in susceptible hosts is indeed universal among the European strains of *B. burgdorferi* s.l. tested so far[23,32,35,86]. WTBE virus, which is transmitted by the same tick species in continental Europe, has not evolved traits that permit persistence in mice, so for some pathogens, persistence might not be achievable, or might be disadvantageous for reasons that remain obscure.

How does the wider host community influence pathogen transmission and survival? There is no evidence that *B. burgdorferi* s.l. regulates the fitness of its hosts, but vertebrate populations are regulated by many other factors. White-footed mice in North America are particularly affected by resource supply ('bottom-up' regulation), resulting in wide intra- and inter-annual fluctuations in their density[66,95]. Increased resource availability is associated with increased reproductive success and survival, whereas density-dependent effects and dispersal can bring population densities back to lower carrying capacities when resources are limited[96]. Increased resource availability can affect many host species, causing complex effects on the dynamics of tick-borne zoonoses[95]. Predation also influences host density and demography ('top-down' regulation)[1,97]. In addition to trophic interactions, cycles of abundance and changes in host structure are affected by seasonal and annual variations of temperature[95,96,98]. These fluctuations in density are inevitably accompanied by variations in the age structure of the host populations and in the rates at which naive hosts are recruited. This is an important element of the population biology of pathogens, including *B. burgdorferi* s.s.[64,66]. However, the net effects of host population fluctuations on the abundance of multi-host vector-borne pathogens are difficult to predict and depend on the conditions, for example, on the levels of host specialism of both the vector and the pathogen[12,99].

Generalism of vectors adds interesting complexity to the life cycle of multi-host pathogens such as *B. burgdorferi* s.l. First, this trait provides opportunities for cross-species transmission[28]. Second, it can allow different host species to additively affect the transmission cycles of vector-borne pathogens, and third, it can have a negative effect on transmission cycles[99]. For *B. burgdorferi* s.s. in the northeastern United States, it has been suggested that the dilution effect operates in species-rich communities[99,100]. According to this concept, hosts that are less efficient than mice as reservoirs for *B. burgdorferi* s.s. 'waste' tick bites as far as the pathogens are concerned[14]. Simple models predict that the infection prevalence of *B. burgdorferi* s.s. in questing ticks is lower in species-rich communities compared with communities dominated by white-footed mice[15]. The infection prevalence falls demonstrably when, in an extreme example, reservoir-competent white-footed mice are replaced by reservoir-incompetent vaccinated mice[94]. In much of Eurasia the principal tick vectors of *B. burgdorferi* s.l. are generalists, but in contrast to the situation in the northeastern United States, most genotypes of *B. burgdorferi* s.l. are specialists. Therefore, it will be interesting to investigate whether dilution and/or amplification effects occur in species-rich host communities of *B. burgdorferi* s.l. in Eurasia[101].

Clonal evolution
The evolution of organisms in the absence of genetic recombination.

Instars
The different developmental stages of ticks.

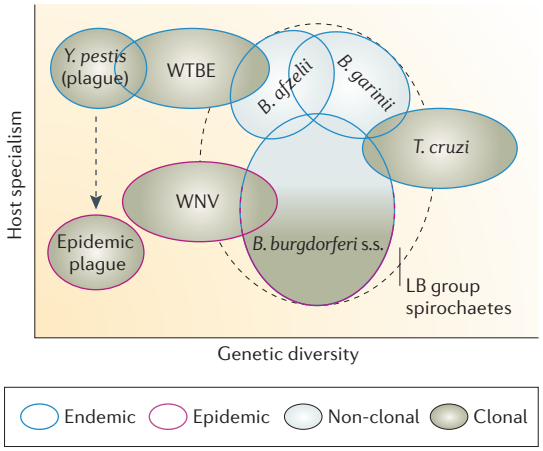


Figure 4 | **The 'taxonomy' of infectious disease dynamics.** Five different zoonotic systems are positioned in a parameter state space spanned by host specialism, genetic diversity, clonality or panmixis, and epidemic or endemic state of the pathogens. These parameters are crucial to understand the evolution and spread of zoonoses. The overlaps in the patterns of transmission dynamics might indicate that at least some common processes operate in these selected zoonotic systems. Under particular ecological conditions, *Yersinia pestis*, the agent of plague, has the potential to become epizootic or even a directly transmitted epidemic disease in humans, indicating that host specialism of this pathogen in its enzootic state is mediated by ecological factors, rather than being an intrinsic property of the bacterium. *B. afzelii*, *Borrelia afzelii*; *B. burgdorferi* s.s., *Borrelia burgdorferi* sensu stricto; *B. garinii*, *Borrelia garinii*; LB, Lyme borreliosis; *T. cruzi*, *Trypanosoma cruzi*; WNV, West Nile virus; WTBE, western tick-borne encephalitis virus.

Multiple niche polymorphism is a theoretical concept that describes the diversification of populations through adaptation to different ecological niches[16]. We hypothesize that multiple niche polymorphism is important for *B. burgdorferi* s.l. population diversity[8,17], because small differences in characteristics among bacterial strains can have greater or lesser effects depending on co-inherited characteristics, such as the effectiveness of immune evasion (FIG. 2). The potential effects of acquired host immunity on the survival of *B. burgdorferi* s.l. should drive the evolution, fixation and transient co-existence of bacterial mutants[6]. These changes might indicate the beginning of adaptation to different ecological niches[29].

Parallels with other vector-borne zoonoses

The development of unifying theories of the evolutionary ecology of pathogens is desirable, but strong support from nature is required to accept these theories for vector-borne zoonotic pathogens or to develop more appropriate models. Given the many vector-borne zoonoses and their possible biological idiosyncrasies, a major concern is how far the features of one system can be generalized to all such systems. Here, a wealth of recent research has allowed us to identify and explore fundamental biological processes in the Lyme borreliosis system, and a first step to address the question of generalization is to identify common biological processes that operate in vector-borne zoonoses. In FIG. 4 we compare *B. burgdorferi* s.l. with other relatively well-understood vector-borne zoonotic pathogens across four epidemiologically and ecologically crucial parameters: host specialism, genetic diversity, clonality and epidemic behaviour[73,92,102,103]. In the parameter space spanned by these variables, *B. burgdorferi* s.l. takes a central place and directly or indirectly overlaps the patterns seen for *Trypanosoma cruzi* (the causative agent of Chagas disease), WTBE virus, WNV and *Yersinia pestis* (the causative agent of plague) in its enzootic state. High genetic diversity, host association and clonality are typical for *T. cruzi*. WTBE virus and *Y. pestis* are less diverse and specialized to rodents. In certain ways, the epidemiology of *B. afzelii* shows at least some parallels with that of WTBE virus, although WTBE virus is even more specialized. Epidemiological parallels are also evident between populations of *B. garinii* and *T. cruzi*. In the northeastern United States, *B. burgdorferi* s.s. is much less specialized than its Eurasian counterparts. This, and the dispersal of this species across the northeastern United States, evokes ecological parallels with WNV, because WNV also uses a wide range of vertebrate hosts, including birds. The degree of clonality of *B. burgdorferi* s.l. can vary over a wide range, perhaps depending on the genomic region measured and on the spatial scale of sampling.

Although based on the best information available, it has to be emphasized that the parallels among the zoonotic systems as shown in FIG 4 are speculative, because they depend on the unspecified scales of the axes and therefore on the way the figure is drawn. These uncertainties point to the urgent need for more empirical data and for the development of algorithms to provide exact measures or numerical indices of these parameters. For example, it is particularly difficult to obtain exact quantitative measures for host specialism of a pathogen, because this is not a simple function of the number of different host species; phylogenetic relationships among host species determine the niche breadth of microparasites and must also be considered[28]. The future challenge is to synchronize field, laboratory and modelling studies to provide insights into mechanisms of evolutionary ecology, whether general or idiosyncratic, within the diverse spectrum of vector-borne pathogens.

Conclusions and future prospects

Biological, process-based simulation models are needed to better illuminate and predict the evolutionary ecology of vector-borne zoonoses. These models should have the power to generate hypotheses of how pathogen traits influence the basic reproduction number, R_0, (BOX 1) in host and vector communities, which should then be tested in the field and laboratory. Overall, we conclude that Lyme borreliosis represents a paradigm system with exciting possibilities for generating and testing

Panmixis
The genetic state of a population with unrestricted gene flow, as opposed to clonality.

hypotheses and models of the evolutionary ecology of vector-borne pathogens, many of which are much less accessible, more virulent and therefore more difficult and expensive to study empirically than Lyme borreliosis. Furthermore, we regard Lyme borreliosis as an ideal system to test whether, and at what level, genotypically defined microbial populations correspond to ecotypes, and to test the biological reality of theoretical species concepts to the satisfaction of taxonomists, ecologists and epidemiologists[29].

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Competing interests statement
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