REVIEW ARTICLE

Ecology of *Borrelia burgdorferi sensu lato* in Europe: transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change

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Abstract

The analysis of different multi-host systems suggests that even hosts that are not capable of transmitting *Borrelia burgdorferi sensu lato* (*s.l.*) to the tick vector, *Ixodes ricinus*, or that are secondary reservoirs for these agents contribute to the intensity of transmission and to the overall risk of Lyme borreliosis, through the process of vector augmentation and pathogen amplification. On the other hand, above certain threshold densities, or in the presence of competition with primary reservoir hosts or low attachment rate of ticks to reservoir hosts, incompetent or less competent hosts may reduce transmission through dilution. The transmission of *B. burgdorferi s.l.* is affected by molecular processes at the tick-host interface including mechanisms for the protection of spirochaetes against the host's immune response. Molecular biology also increasingly provides important identification tools for the study of tick-borne disease agents. *Ixodes ricinus* and *B. burgdorferi s.l.* are expanding their geographical range to northern latitudes and to higher altitudes through the effects of climate change on host populations and on tick development, survival and seasonal activity. The integration of quantitative ecology with molecular methodology is central to a better understanding of the factors that determine the main components of Lyme borreliosis eco-epidemiology and should result in more accurate predictions of the effects of climate change on the circulation of pathogens in nature.

Introduction

The causative agents of Lyme borreliosis are tick-transmitted spirochaetes belonging to the *Borrelia burgdorferi* species complex (*B. burgdorferi sensu lato* (*s.l.*)) in which there are known to be at least 19 genospecies with a worldwide distribution (Table 1). Whereas in North America only one genospecies, *B. burgdorferi sensu stricto* (*s.s.*), is a confirmed pathogen of humans, in Europe, four additional pathogens, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia spielmanii* and *Borrelia bavariensis*, also occur. Three further European genospecies, *Borrelia valaisiana*, *Borrelia lusitaniae* and the recently described *Borrelia finlandensis* (Casjens *et al.*, 2011), are of uncertain pathogenicity. The primary vector tick throughout Europe is *Ixodes ricinus* but in some of the Baltic States another tick, *Ixodes persulcatus*, the main vector in Asia, is sympatric with *I. ricinus*. Some other European tick species, such as the hedgehog tick, *Ixodes hexagonus*, have been shown to be vector-competent for *B. burgdorferi s.l.* (Gern *et al.*, 1991), but are rarely if ever involved in transmission to humans, their main significance being their contribution to the natural circulation of the spirochaetes.

As each genospecies of these tick-transmitted pathogens shows predilections for particular hosts and are also associated with different clinical manifestations, it is apparent that the eco-epidemiology of Lyme borreliosis is extremely complex (Gern, 2008). In fact, the mechanisms underlying the maintenance and transmission of *B. burgdorferi s.l.* in natural cycles have still to be clarified. From the point of view of public health, ecology may clarify the causes of the observed variations, in space and time, in

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Table 1. List of *Borrelia burgdorferi sensu lato* genospecies*

Genospecies	Distribution
B. afzelii	Europe
B. americana	North America
B. andersonii	North America
B. bavariensis	Europe
B. bissettii	Europe, North America
B. burgdorferi	Europe, North America
B. californiensis	North America
B. finlandensis[†]	Europe
B. carolinensis	North America
B. garinii	Europe, Asia
B. kurtenbachii	North America
B. lusitaniae	Europe
B. japonica	Japan
B. sinica	China
B. spielmanii	Europe
B. tanukii	Japan
B. turdi	Japan
B. yangtze[‡]	China
B. valaisiana	Europe, Asia

*Updated from EUCALB website (http://meduni09.edis.at/eucalb/cms/index.php?option=com_content&task=view&id=58&Itemid=91).
[†]Casjens *et al.* (2011).
[‡]Chu *et al.* (2008).

the density of host-seeking infected *I. ricinus* nymphs (DIN) and in nymph infection prevalence (NIP), that directly affect the risk of Lyme borreliosis for humans and some domestic animals (Jouda *et al.*, 2004a). By studying the influence of habitat on the transmission cycle of *B. burgdorferi s.l.*, ecological research provides the basis for landscape epidemiology (Reisen, 2010).

Although *I. ricinus* (Fig. 1) spends only a small fraction of its lifespan on the host, the tick's development and reproduction, and the transmission of *B. burgdorferi s.l.*, strictly depend upon the consumption of vertebrate blood. Based on a classification of tick-borne zoonoses proposed by Mather & Ginsberg (1994), Lyme borreliosis can be considered as a class III-zoonosis, where transmission occurs between one tick vector, *I. ricinus* in western Europe, and multiple vertebrate reservoir hosts, whereas clinical consequences of infection only take place in dead-end hosts, such as humans. Different host species vary in their ability to acquire *B. burgdorferi s.l.* from infected ticks and to subsequently transmit it to other, uninfected ticks (reservoir competence), and in their suitability to serve as hosts for the vector's life stages (Gern, 2008). As a consequence, host population abundance and composition are key factors for *B. burgdorferi s.l.* maintenance and intensity of transmission.

Mathematical models have been used to facilitate understanding of the complex interactions between tick and host populations. Indeed, simulation of different scenarios allows study of hypotheses generated in field research and

provides insight into apparently counterintuitive or contrasting empirical observations. Through the identification and evaluation of key parameters in transmission, models are particularly suited to synthesize current knowledge and to indicate priorities for future research.

In addition to quantitative relationships among host and vector populations, molecular processes, acting at the interfaces between vertebrate hosts, tick vectors and spirochaetes, greatly affect *B. burgdorferi s.l.* transmission. For example, the vertebrate host specificity of European *B. burgdorferi s.l.* genospecies is mediated by host immunity through an antibody-independent activation of the alternative complement pathway (Kurtenbach *et al.*, 2002b). New insight into host specificity has been made possible by the development of molecular techniques to detect and characterize host DNA in host-seeking ticks, which permits identification of reservoir or incompetent hosts (Gray *et al.*, 1999; Pichon *et al.*, 2003, 2005; Humair *et al.*, 2007; Morán Cadenas *et al.*, 2007a). However, the associations between genospecies and hosts are not absolute, may vary geographically, and the molecular tools required for host specificity analysis need to be further developed (Gern, 2008). One of the major research tasks is the integration of molecular approaches with modelled relationships between populations of hosts and vectors.

The importance of climate effects on *B. burgdorferi s.l.* transmission cannot be overemphasized. This is because most *I. ricinus* development and activity take place off-host and therefore under the direct influence of temperature and humidity at the microclimatic level. Furthermore, vegetation cover and animal host populations are affected by climate, as well as by interactions among all of the components of the cycle. The study of the contribution of climate change to the observed expansion of the geographical range of Lyme borreliosis is therefore a particularly timely and appropriate objective of ecological research.

The purpose of this review is to provide a synthesis of the current status of ecological research on *B. burgdorferi s.l.* and to identify promising areas for further studies. The main focus of the review is on the role of multiple hosts as a major source of complexity in the agent's transmission dynamics. Key aspects of molecular biology and molecular tools are identified and considered in an ecological context. Finally, the effects of climate change on ticks and tick-borne diseases are discussed as an example of a complex problem in need of the integrated application of such knowledge and tools.

Transmission dynamics of *B. burgdorferi s.l.*

Ixodes ricinus can feed on virtually all vertebrates sharing its habitat (Fig. 1). Larval *I. ricinus* are mainly found on
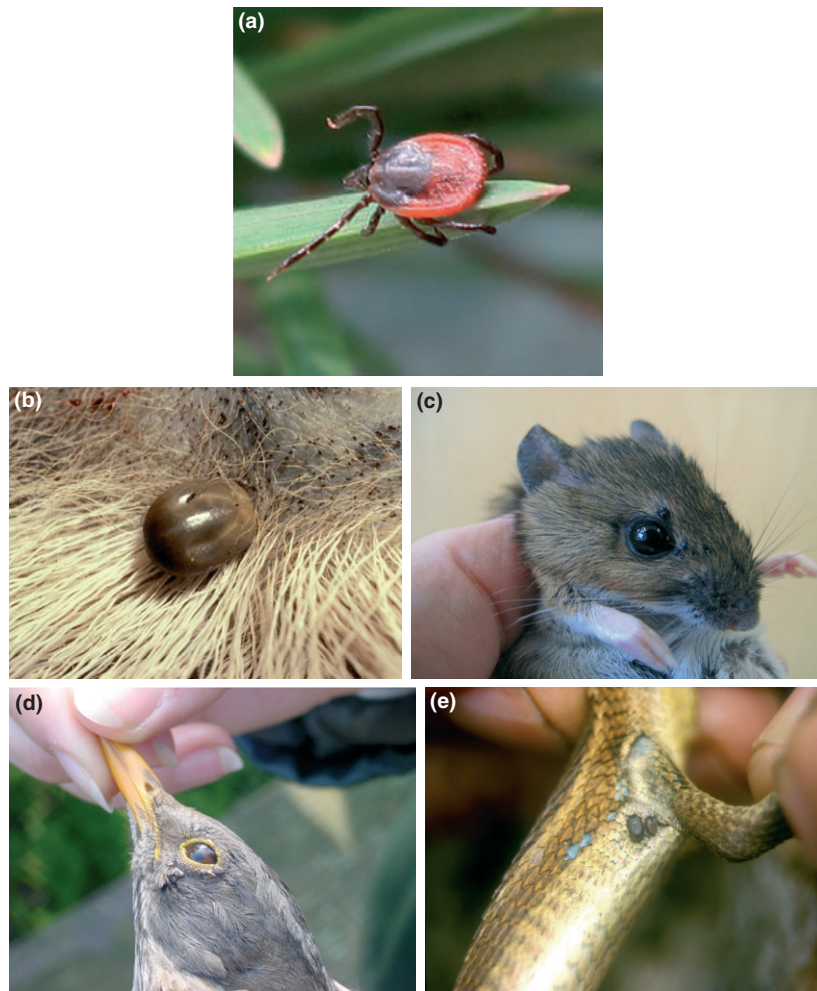
Fig. 1. *Ixodes ricinus.* (a) unfed adult female © L. Gern, (b) engorged female on deer © Anon, EUCALB, (c) larvae and nymphs on *Apodemus* spp. © L. Gern & O. Rais (d) nymphs on *Turdus* spp. © P.G. Meneguz (e) nymph on lizard © A. Estrada-Peña.

small mammals, nymphs on medium-sized mammals, birds and reptiles, and adults mostly on large animals such as ungulates (Fig. 2). The maintenance of *B. burgdorferi s.l.* depends on small and medium-sized mammals, birds and reptiles, acquiring the infection from nymphs and subsequently transmitting it to larvae (Fig. 3). Wild and domestic ungulates, such as deer and cattle, are not considered competent reservoir hosts, although they serve as hosts for large numbers of ticks and contribute significantly to the vector population. The role of sheep is still uncertain. Epidemiological studies suggest that sheep are not important reservoir hosts (Gray *et al.*, 1995), whereas *in vitro* studies demonstrated that some Lyme borrelia genospecies are less susceptible to complement-mediated killing by sheep serum compared with sera from cattle or deer (Kurtenbach *et al.*, 2002a; Bhide *et al.*, 2005).

Although transovarial transmission of spirochaetes, from female ticks to larvae, is rare (Bellet-Edimo *et al.*,

2005), this mode of transmission might contribute significantly to the maintenance of the infection in small rodents. In fact, mice and voles serve as hosts for relatively large numbers of larvae (> 10 per rodent) and therefore transmission could sometimes occur even at larval infection levels as low as *c.* 1% (de Boer *et al.*, 1993). In some areas, the frequency of nymphal bites on small rodents might be too low (0.0074 bites per day on average) to ensure maintenance of the transmission cycle without larval transmission (Randolph & Craine, 1995).

The precise role of hares (*Lepus* spp.) and hedgehogs (*Erinaceus europaeus*) in the circulation of *B. burgdorferi s.l.* is unknown, but as they are reservoir competent for several genospecies (Jaenson & Tälleklint, 1996; Gern *et al.*, 1997), newly acquired alimentary infections in adult female ticks, which can feed to repletion on these animals, may result in a greater chance of transovarial transmission and thus infected larvae. Badgers (*Meles*
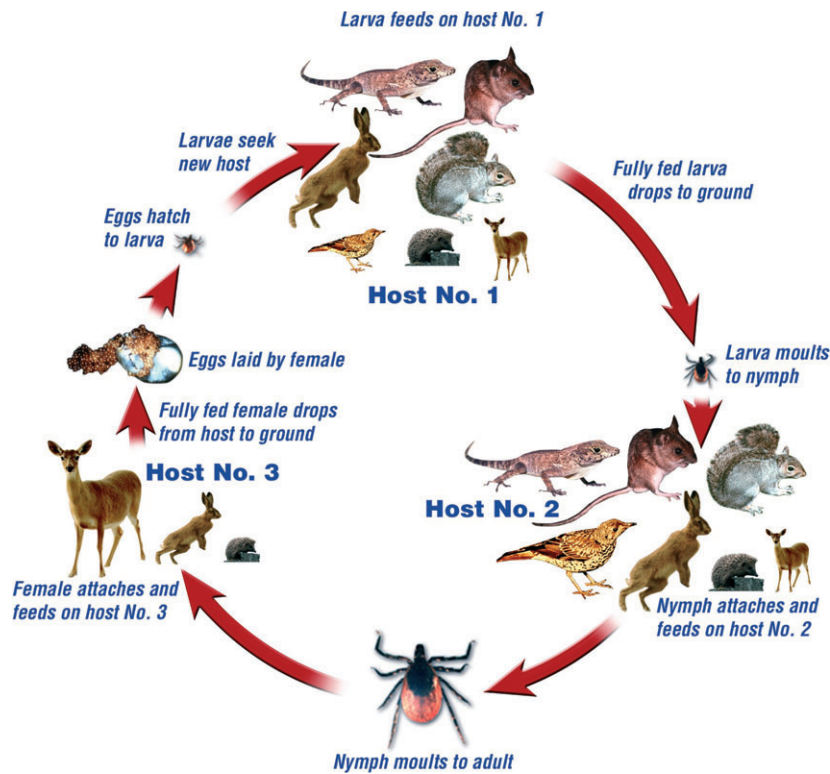
Fig. 2. Life cycle of *Ixodes ricinus*. Each stage takes approximately a year to complete. The relative size of the animals approximates their significance as hosts for the different life-cycle stages in a typical woodland habitat. Reproduced from Eisen & Lane (2002) with permission from CABI Publishing.

meles) and red foxes (*Vulpes vulpes*) may theoretically also have such a role in that adult *I. ricinus* can feed successfully on these animals, but although *B. burgdorferi s.l.* has been isolated from skin samples (Heidrich *et al.*, 1999; Gern & Sell, 2009) and blood-meal analysis studies suggest that foxes can transmit the infection to ticks (Morán Cadenas *et al.*, 2007a), there is no direct evidence that these host species are reservoir competent.

Systemic infection of vertebrates, followed by a relatively long duration of infectivity for ticks [many months in some cases (Gern *et al.*, 1994)], is the main route of transmission of *B. burgdorferi s.l.* from reservoir hosts to ticks. Under these circumstances, transmission is most likely if infectious *I. ricinus* nymphs are active in spring, and susceptible larvae are active in the summer of the same year, so that reservoir hosts are first infected by nymphs and then transmit to larvae.

Borrelia burgdorferi s.l. may also be transmitted among ticks feeding in close proximity on a host's skin (co-feeding transmission), in the absence of systemic infection (Gern & Rais, 1996). Transmission among co-feeding infected nymphs and uninfected larvae may occur during synchronous infestation of vertebrates and is favoured by clustering of ticks on the host skin. Therefore, the tempo-ral pattern of host-seeking tick activity affects different routes of transmission. Co-feeding transmission, although possibly of less ecological significance than systemic transmission, may lead to the re-evaluation of the contribution to the transmission cycle of hosts such as ungulates, which are generally considered to be incompetent reservoirs (Kimura *et al.*, 1995; Ogden *et al.*, 1997; Pichon *et al.*, 2005). However, the role of co-feeding transmission in nature has probably not yet been fully revealed. In fact, it has been recently shown for *B. afzelii* that co-feeding transmission contributes to the promotion and maintenance of *Borrelia* genotype diversity within local tick populations (Pérez *et al.*, 2011) and may therefore be of more significance than previously thought.

The role of a vertebrate species in the transmission of *B. burgdorferi s.l.* results from the combination of host infectivity to susceptible ticks, host population abundance and tick load (Mather *et al.*, 1989; Brunner & Ostfeld, 2008). Host infectivity can be estimated as the fraction of uninfected larvae that acquire the infection after feeding on a host of a certain species. Its extent and duration may vary among hosts and among *B. burgdorferi s.l.* strains and can be affected by the degree of exposure of a host to infectious tick bites and to the degree and

Fig. 3. Transmission cycle of pathogenic *Borrelia burgdorferi sensu lato* genospecies. The size of coloured circles indicates the relative prevalence of the genospecies. © Anon, EUCALB.

duration of infestation by uninfected ticks (Gern *et al.*, 1994). The successful survival and moulting of ticks from one stage to another after the uptake of the blood meal is affected by host immunity (Kurtenbach *et al.*, 1994; Dizij & Kurtenbach, 1995; Humair *et al.*, 1999), and this also contributes to a host species' role in the transmission of *B. burgdorferi s.l.*

Host species population abundance and composition vary across locations. Certain hosts are habitat generalists, whereas the distribution of other species, such as squirrels, is limited to favourable habitats. Furthermore, host abundance may undergo temporal variations. For example, wild mice, such as *Apodemus sylvaticus*, are habitat generalists, but their populations undergo marked temporal fluctuations, affecting the transmission of *B. afzelii* in Europe (see section on multi-host system 3).

The loads and patterns of *I. ricinus* infestation vary widely among hosts. The distribution of ticks among hosts belonging to the same species is typically aggregated, meaning that the majority of ticks feed on a relatively small fraction of the host population. Aggregation of ticks depends on individual host factors, such as immunity and behaviour, as well as on the spatial distribution of host-seeking ticks. The predilections of different tick stages for different hosts and variations in tick distri-

bution patterns among hosts of the same, and of different species, therefore have consequences for the transmission of *B. burgdorferi s.l.* (see below).

In Europe, *B. burgdorferi s.l.* comprises several species (genospecies) of spirochaetes characterized by differences in their reservoir hosts and in the clinical consequences of their infection to humans. *Borrelia garinii* is associated with neuroborreliosis and with birds; though, *B. garinii* serotype 4, now known as *B. bavariensis* (Margos *et al.*, 2009), utilizes mice as reservoirs (Huegli *et al.*, 2002). *Borrelia burgdorferi* ss and *B. afzelii* are found in small- and medium-sized mammals (as well as birds in the case of *B. burgdorferi s.s.*) and cause joint and skin lesions. *Borrelia spielmanii*, the last pathogenic genospecies identified in Europe, was found in two species of dormouse (Richter *et al.*, 2006) and seems to be associated with skin lesions. The role of other genospecies in clinical Lyme borreliosis remains to be clarified. Among these, *B. valaisiana* is associated with birds (Humair *et al.*, 1998) and *B. lusitaniae* with lizards (Dsouli *et al.*, 2006), although the latter has also been found in birds (Poupon *et al.*, 2006) and mice (de Carvalho *et al.*, 2010).

The prevalence of *B. burgdorferi s.l.* infection in host-seeking *I. ricinus* and the frequency of each genospecies vary over short distances. In general, the relative

abundance of reservoir hosts for different genospecies is an important factor affecting such variations; however, underlying ecological mechanisms still need to be clarified. Moreover, the vector competence of *I. ricinus* probably varies across locations, and one study has suggested that different populations of ticks may vary in susceptibility to the genospecies *B. afzelii* (Estrada-Peña *et al.*, 1998).

Transmission dynamics of *B. burgdorferi s.l.* in multi-host systems

The effects of the combination of host species, characterized by different patterns of tick infestation and reservoir competence, are a central objective of research on the transmission dynamics of *B. burgdorferi s.l.* To highlight the most important findings of studies in this field, we distinguish three multi-host systems and corresponding scenarios regarding *B. burgdorferi s.l.* transmission:
(1) Multi-host system 1: which includes two types of animal species: competent reservoir hosts for spirochaetes, such as mice, and incompetent reservoirs, such as deer, that on the other hand, serve as host for all tick stages, including adults.
(2) Multi-host system 2: including different reservoir hosts characterized by variable reservoir competence for the same *B. burgdorferi s.l.* genospecies; here, a primary reservoir host can be identified based upon high infectivity to ticks, together with secondary reservoirs characterized by a lower infectivity.
(3) Multi-host system 3: including animal species acting as competent reservoirs for different *B. burgdorferi s.l.* genospecies, this system is peculiar of the European situation.

Multi-host system 1

Here, ticks feed on two types of hosts: H1, competent reservoirs for *B. burgdorferi s.l.* and hosts for immature ticks; H2, incompetent reservoirs for *B. burgdorferi s.l.* and main hosts for adult ticks. This is a situation that depends on the assumption that the main hosts for adult ticks, such as wild and domestic ungulates, are not able to transmit the infection to feeding ticks. Furthermore, ungulates may reduce infection prevalence in host-seeking nymphs (NIP) by feeding relatively large proportions of larvae that moult to noninfected nymphs and by diverting ticks from competent reservoirs (*encounter reduction*, cf. Keesing *et al.*, 2006). Under these circumstances, ungulate population density would be negatively associated with *B. burgdorferi s.l.* transmission, corresponding to a *dilution effect*. Conversely, large populations of reservoir hosts feeding the majority of larval ticks would be associated with intense transmission.

While several field studies in Europe supported the hypothesis of the incompetence of deer and other ungulates as reservoirs for *B. burgdorferi s.l.*, variable results were found on the effect of ungulate populations on spirochaete transmission (Gray *et al.*, 1992, 1995; Jaenson & Tälleklint, 1992; Matuschka *et al.*, 1993; Tälleklint & Jaenson, 1994; Pichon *et al.*, 1999), and in certain cases the *dilution effect* could not be demonstrated. Moreover, ungulates serve as hosts for adult ticks and are therefore largely responsible for the density of host-seeking ticks (*vector augmentation*). Consequently, when deer are uncommon, tick populations are reduced and transmission is less intense, whereas dilution comes into play at high deer densities (Gray *et al.*, 1992; Rizzoli *et al.*, 2002).

The complex relationships between host population densities and transmission of *B. burgdorferi s.l.* have been studied by mathematical models. Specifically, the effects of combined variations of population densities of competent (H1) and incompetent hosts (H2) on *B. burgdorferi s.l.* were simulated, and results were visualized by threshold curves, separating combinations of host densities that maintain ticks and/or the pathogen (basic reproduction number, $R_o > 1$), from those that result in them dying out ($R_o < 1$). This approach was applied by Bowers & Turner (1997) to directly transmitted agents, whereas Norman *et al.* (1999) identified threshold curves for the persistence of louping-ill virus, transmitted by *I. ricinus*, at different combinations of population densities of viraemic and nonviraemic hosts.

For *B. burgdorferi s.l.*, Fig. 4 shows a threshold curve corresponding to values of $R_o = 1$ at different combinations of the density of H1 (competent reservoir hosts, *x*-axis) and H2 (incompetent hosts e.g. ungulates, *y*-axis) (Rosà *et al.*, 2003). R_o is < 1 left of the threshold curve, meaning that *B. burgdorferi s.l.* transmission would not occur at the corresponding combinations of densities of hosts, whereas $R_o > 1$ to the right of the curve, where *B. burgdorferi s.l.* is maintained. This curve shape, with a convexity to the left, is the key characteristic of this multi-host system. In fact, the convexity identifies the optimal density of H2 (deer) that would allow transmission even at relatively low densities of H1 (mice). Below such H2 density, ticks would be too scarce and R_o would therefore be reduced; consequently, increasing H2 density within this range would favour transmission. Conversely, when the density of H2 is above the convexity, increasing H2 density would reduce transmission through a dilution effect; this would be more evident when H1 are relatively rare. Other conditions that might make transmission more sensitive to the dilution effect exerted by H2 include low attachment rates of nymphs on H1 (*c.* 0.01 bites per day per mouse).

An early study on the effects of ungulate density showed that tick abundance inside a deer fence that
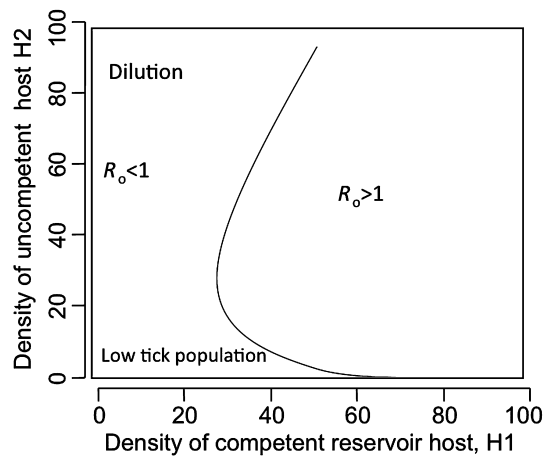
Fig. 4. Threshold curve ($R_o = 1$) for the transmission of *Borrelia burgdorferi sensu lato* at varying combinations of population densities of two vertebrate species: H1, a competent reservoir host and H2, an incompetent reservoir for the spirochaetes, serving as host for all stages of *Ixodes ricinus*. The pathogen does not persist ($R_o < 1$) when population densities of incompetent hosts (H2) and, consequently, of ticks are too low; at densities of competent hosts H1 between 30 and 60, increasing H2 density favours transmission ($R_o > 1$) through vector augmentation. On the other hand, when H2 density is above a certain level, dilution occurs and the pathogen does not persist ($R_o < 1$) (modified, from Rosà *et al.*, 2003).

enclosed a large herd of fallow deer (*Dama dama*) in an Irish forest was significantly higher than outside the fence, but that the infection rate of nymphs with *B. burgdorferi s.l.* was much lower than outside the fence (Gray *et al.*, 1992). A more detailed and controlled fence study was conducted by Perkins *et al.* (2006), who reported that within small areas (< 1 ha) from which deer were excluded, the number of host-seeking nymphs was greater than outside, where deer had access. It was concluded that the effect of deer exclosure depends on the size of exclusion areas and the movements of mice between the inside and outside of the exclosure. Large exclosures result in the eventual reduction in ticks within them, because of the absence of deer, whereas small exclosures show an increase in host-seeking ticks. This latter observation was interpreted as being due to transfer of ticks across the fence by rodents, causing accumulation of host-seeking ticks where deer are not available to remove them. Additionally, several rodents captured within one small exclosure carried antibodies to tick-borne encephalitis (TBE) virus (also transmitted by *I. ricinus*), whereas none were seropositive in control areas outside the exclosures. These results suggest that within small patches of habitat the risk of tick-borne disease might be elevated by the greater abundance of infected host-seeking ticks. Such an effect could explain the observations of high abundance of *Ixodes scapularis* infected with *B. burgdorferi s.l.*

in fragmented habitat in the USA (Allan *et al.*, 2003), and similar mechanisms might be operating in a small city park in coastal Tuscany, Italy, where human cases of Lyme borreliosis were alleged to occur and where *B. garinii* was found in host-seeking *I. ricinus* (Stefanelli *et al.*, 1994). As no ungulates were present in the park, infectious nymphs might have originated from birds roosting in the park, and dropping engorged larvae. Despite such observations on disease risk in small habitats, the interactions between hosts, vectors and pathogens are evidently very complex and further studies on this topic are required.

Studies in the USA (Rand *et al.*, 2004) showed that the degree of deer reduction required for effective reduction in risk is uncertain, which is to be expected in view of the complex relationships revealed by models. It was concluded that deer control should be maintained indefinitely. In fact, when deer density is low, increases in deer numbers would lead to an increase in *B. burgdorferi s.l.* transmission (Fig. 4). Also, increased numbers of host-seeking nymphs and adults would be observed for 2–3 years after deer reduction, and questing ticks could be characterized by relatively high infection prevalence (e.g. > 10%).

As a general conclusion on multi-host system 1, the potential dilution effect of wild and domestic ungulates on *B. burgdorferi s.l.* transmission and the role of these host species in vector augmentation make it very difficult to predict NIP based solely on their abundance.

Multi-host system 2

This ecological transmission system is more common and therefore more studied in North America where several host species, including small- and medium-sized mammals and birds, may serve as reservoirs for *B. burgdorferi* ss. In this system, the white-footed mouse (*Peromyscus leucopus*) is considered to be the *primary competent reservoir*, and this conclusion is based upon the great infectivity of mice to feeding larvae of the main North American tick vector, *I. scapularis* [probability of infection of larvae after feeding on mice = 0.463 (Mather *et al.*, 1989)], together with the relatively high larval burdens (> 10 per host). Moreover, *P. leucopus* is a habitat generalist and can thrive even in habitats that were heavily modified by humans, and where other hosts for *I. scapularis* are rare or absent. This multi-host system is also relevant in Europe, where *Apodemus* spp. mice occupy an ecological niche that is similar to *P. leucopus* in North America, whereas other host species, including red squirrels, may be *secondary B. burgdorferi s.l. reservoirs* (Humair & Gern, 1998). A further dimension is added by the significant reservoir competence of the introduced North American

sciurids, grey squirrels (*Sciurus carolinensis*) (Craine *et al.*, 1997) and chipmunks (*Tamia* spp.) (Vourc'h *et al.*, 2007).

As for multi-host system 1, even in this case, conclusions on the effects of the composition of the host populations on the intensity of *B. burgdorferi s.l.* transmission cannot be generalized to all ecological settings. In fact, field studies and subsequent theoretical models from the North Eastern USA suggested that the abundance of less efficient hosts and therefore increased biodiversity in habitats where mice serve as primary reservoirs lead to a dilution of *B. burgdorferi s.l.* infection (LoGiudice *et al.*, 2003). Conversely, Ogden & Tsao (2009) suggested that the consequence of biodiversity is mostly towards amplification of transmission rather than dilution. The main reason proposed is that tick abundance is strongly correlated with that of vertebrate hosts (regardless of their role as reservoirs for *B. burgdorferi s.l.*), and therefore increased host density would lead to increased tick density and more efficient transmission. Therefore, in both multi-host 1 and multi-host 2 systems, the potential dilution role of noncompetent or less competent hosts is counteracted by their role in vector augmentation. Consequently, within a wide range, increasing host density would increase the numbers of infected ticks. Dilution would only occur in the following conditions: much greater attachment rates for nymphs on secondary reservoir hosts than on mice, density-dependent mortality of ticks on secondary hosts and direct competition meaning that secondary host density would negatively affect mouse density.

From a more general conceptual point of view, Ogden & Tsao (2009) concluded that transmission of *B. burgdorferi s.s.* is, to a large extent, a density-dependent phenomenon, meaning that development and reproduction of ticks and consequently transmission of infection are greatly conditioned by the availability of vertebrate hosts. However, there is also a frequency-dependent component in transmission represented by the effect of prevalence of infection in hosts and in ticks. To further increase complexity, the relative role of different reservoir species may vary with time. Field observations in the upper mid-west USA, for example, suggested that when mice are at a very low population density (< 8 mice per ha), the presence of other species such as chipmunks (increased biodiversity) might compensate and sustain the transmission cycle (Slajchert *et al.*, 1997).

Multi-host system 3

In Europe, multiple genospecies of *B. burgdorferi s.l.* are transmitted by *I. ricinus* and show some degree (even if not absolute) of specificity to different vertebrate species serving as reservoir hosts. In a previous study carried out across Europe (Saint-Girons *et al.*, 1998), *B. garinii* was found to be the most widespread genospecies in host-seeking ticks, suggesting a major role of birds in the eco-epidemiology of Lyme borreliosis. It was also concluded that prevalence of infection increased from western to eastern Europe. More recently, a meta-analysis of European publications showed that *B. garinii* and *B. afzelii* were the most common *Borrelia* species and that tick infection prevalence was greatest in central European countries (Rauter & Hartung, 2005). It is, however, acknowledged that the relative frequency of different genospecies varies in different regions in Europe. At the local level, field observations showed that the prevalence of *B. burgdorferi s.l.* and of the different genospecies in host-seeking ticks vary even within a small geographical scale.

In the multi-host system 3, the composition of host populations not only affects the intensity of transmission of *B. burgdorferi s.l.* but it also determines the relative frequency of different genospecies. Under these circumstances, vertebrate species may serve as primary reservoir hosts for particular genospecies and, at the same time, they may affect the transmission of other genospecies, through dilution, or vector augmentation.

Complex interactions among host population dynamics and habitat may lead to dominance, maintenance or disappearance of specific genospecies under different scenarios. Moreover, in certain areas, several genospecies may simultaneously thrive, whereas at other locations, one genospecies might be dominant while the others are rare. As an example, in a sub-Mediterranean habitat on Le Cerbaie Hills, in Tuscany, *B. lusitaniae* was the dominant genospecies in host-seeking *I. ricinus*, while other genospecies were rarely found (Bertolotti *et al.*, 2006). Field observations suggested that lizard abundance might explain dominance of *B. lusitaniae* on Le Cerbaie. Furthermore, lizards were shown to feed approximately the same numbers of larvae as mice (on average, 4.2 and 3.5 larvae per lizard and mouse, respectively, in 2005), but greater numbers of nymphs (0.47 per lizards against three nymphs on 53 mice). It was concluded that an environment suitable to lizards, but with enough moisture to allow development and activity of *I. ricinus*, might create favourable conditions for the dominance of *B. lusitaniae* (Amore *et al.*, 2007). It was also hypothesized that lizards might interfere with the transmission of other genospecies through dilution. The presence of *B. afzelii* in *I. ricinus* larvae and in tissues collected from *Apodemus* spp. mice seemed to be affected by habitat type, mouse population density and levels of infestation of mice by nymphs. This was based on the observation that *B. afzelii* was only found in *I. ricinus* larvae feeding on mice that were captured in a relatively dry upland site, where mouse density

and infestation by nymphs were relatively great. On the other hand, *B. afzelii* was not found in mouse tissues and in attached larvae in a relatively humid bottomland habitat.

Although field observations in Le Cerbaie were limited in space and in time and therefore the study design was not representative of the entire area, a simple mathematical model was constructed to examine hypotheses concerning the ecological processes underlying the transmission dynamics of these *B. burgdorferi s.l.* genospecies. The model's specific objectives were to gain insight into conditions for the dominance of *B. lusitaniae* and to explore different transmission scenarios, under which *B. afzelii* may fail to be maintained or be transmitted at low levels (Ragagli *et al.*, 2011). The model results confirmed that *B. lusitaniae* was well established in the study area ($R_o > 1$), in association with the presence of lizards serving as hosts for large numbers of nymphs in spring 2006 (mean number of nymphs per lizard varying from 3.3 to 6.0 depending on habitat) and of larvae in the summer (mean varying from 4.9 to 7.4 larvae per lizard). On the other hand, the model also showed that persistence of *B. afzelii* ($R_o > 1$) was only possible when mouse population density and nymph infestation of mice were at the maximum limits of the field estimates in the study area (116.1 mice per ha, and 1.7 nymphs per mouse were estimated in upland habitat). The model also supported the hypothesis that fluctuations in time or between habitats of these two parameters may lead to extinction of *B. afzelii*, because R_o may decrease to values below 1. Therefore, mouse population fluctuations and low attachment rates of nymphs to mice were key factors affecting transmission intensity and genospecies composition. Indeed, through the application of next-generation matrix models in a previous study, survival probability from feeding larva to feeding nymph, including nymph attachment, was identified as the parameter that most strongly affected R_o for *B. burgdorferi s.l.* (Hartemink *et al.*, 2008).

It is worth noting that *B. afzelii* was found in the drier habitat type in the study area and where the collection of host-seeking ticks by dragging yielded lowest numbers of nymphs per 100 m (Bisanzio *et al.*, 2008). A possible explanation was the different host-seeking behaviour of nymphs in different habitats. For example, the lower humidity in the upland could determine the displacement of nymphs to seek hosts very low near the ground to avoid desiccation (Randolph & Storey, 1999), or a greater host-seeking activity of nymphs during the night, when humidity is greater. Both hypothetical factors would reduce the probability of collecting nymphs by dragging, but would favour the encounter of nymphs with mice, which are mostly active during the night and dwell near the ground. Conversely, it might be suggested that, in the

relatively humid bottomland, nymphs seek hosts low down in the vegetation and are more likely to attach to small animals, during the warmer part of the day, when mice are inactive and lizards are active. Humid habitat in warm climate might therefore favour *I. ricinus* infestation of lizards, creating conditions for the dominance of *B. lusitaniae* over other genospecies.

The results from Le Cerbaie were different from those obtained in Germany (Richter & Matuschka, 2006), where *B. afzelii* and *B. lusitaniae* coexisted, probably due to a generally greater level of *B. burgdorferi s.l.* transmission. The sub-Mediterranean, relatively dry habitat in Le Cerbaie, is not optimal for *I. ricinus*, a tick species that is sensitive to desiccation. This, together with a very low roe deer density in the area (< 2 per 100 ha), might lead to a relatively scarce tick population in comparison with Central European sites. Under these circumstances, only one genospecies, *B. lusitaniae*, might thrive whereas *B. afzelii* might be sporadic. In general, this would suggest the hypothesis that a large *I. ricinus* population favours the coexistence of multiple *B. burgdorferi s.l.* genospecies, whereas less favourable conditions for the tick vector might be compatible with the maintenance of only one genospecies.

Geographical variations in host-seeking and in attachment behaviour among *I. ricinus* populations are the objects of growing research interest to explain the variable transmission dynamics of *B. burgdorferi s.l.* genospecies. The rarity of *B. afzelii* in the UK and Ireland, as an example, might be a result of the relative failure of nymphs to attach to mice despite much contact between them, possibly due to inherent tick behavioural characteristics (Gray *et al.*, 1999). Alternatively, tick populations may show regional differences in their susceptibilities to various *B. burgdorferi s.l.* genospecies (Estrada-Peña *et al.*, 1998).

Aggregation of ticks among hosts

As for many parasites, the distribution of ticks among vertebrate hosts is neither homogeneous nor random. Most individuals in a host population carry relatively few ticks (or none in certain cases), and the majority of ticks are aggregated on a small fraction of individuals. Such an aggregated distribution of vectors causes major differences in the contribution of individual hosts to the transmission of tick-borne agents. This is in agreement with the general conclusion that individuals contribute differently to the maintenance and spread of infectious agents, including those that are transmitted by vectors, and that a relatively small proportion of hosts is responsible for the majority of transmission events or for feeding the majority of vectors (Woolhouse *et al.*, 1997).

There are several factors that can be hypothesized as causes of increasing tick numbers on individuals belonging to the same species. These include individual characteristics, such as immunity, movements, home range and grooming activity, as well as the patchy distribution of host-seeking ticks in the environment. Host-seeking behaviour by ticks may also affect host burdens.

The effect of aggregation of *I. ricinus* nymphs and larvae on the same host at the same time (aggregated, coincident distribution) is a well-known factor in the nonsystemic transmission of TBE virus among ticks feeding on the same individual host (Randolph *et al.*, 1999). In field studies carried out in Italy, some degree of co-infestation by *I. ricinus* nymphs and larvae, some of which were infected with *B. garinii* or *B. valaisiana*, was observed on blackbirds (*Turdus merula*) in summer (Mannelli *et al.*, 2005), and this would allow co-feeding transmission to take place alongside the systemic transmission of certain genospecies that occurs in this reservoir host (Humair *et al.*, 1998). Conversely, no evidence of co-infestation was found in lizards in Italy (Amore *et al.*, 2007), suggesting that systemic infection of these hosts is the dominant transmission route for *B. lusitaniae*, which was found in both lizards and the immature ticks feeding on them. The aggregation of both nymphs and larvae on the same hosts also favours systemic transmission if sufficient time elapses between activity of the two tick stages to allow development of the systemic infection in the host and subsequent infection of larvae.

Aggregation of different tick stages on the same individual hosts may not be the rule, because of interacting factors associated with heavy burdens of ticks on certain hosts (Brunner & Ostfeld, 2008). Host sex, for example, might be associated with tick burdens. In fact, sexually active male small rodents might be more exposed to tick infestation during the spring peak of nymphal activity, because of increased contact with ticks while searching for mates and to immuno-depression associated with intra-species competition. Elevated testosterone levels in mice may also increase susceptibility to ticks, thus contributing to the aggregated distribution of the ticks (Hughes & Randolph, 2001). Female rodents, on the other hand, are more likely to be susceptible to tick infestations later in the season, during lactation, when immune suppression may occur because of the combined effects of prolactin hormone and stress.

Aggregation of ticks among hosts is a key aspect to be taken into account when analysing field data. In the statistical analysis of tick infestation load of individual hosts, a negative binomial distribution is widely used to take into account aggregated distribution, which leads to *overdispersed* counts. Furthermore, using negative binomial distributions in statistical modelling, an interpretable

parameter measuring aggregation can be obtained. Other aggregation parameters include mean crowding and patchiness (less affected by the mean burden) (Kitron *et al.*, 1991). Similar approaches can be used to analyse the distribution of host-seeking ticks in the environment (Bisanzio *et al.*, 2008). When the number of individual animals or geographical locations with no ticks exceeds the frequency that can be accommodated by the negative binomial distribution, the so-called zero-inflated mixture models can be used.

Network analysis provides a particularly suitable set of tools to analyse and simulate the effects of aggregated distributions of ticks on tick population dynamics and transmission of pathogens. Here, each individual (or group of individuals) can be envisioned as a node, whereas links among nodes represent potentially infectious contacts (Pastor-Satorras & Vespignani, 2001). Network analyses have been profitably used to simulate the spread of directly transmitted infections in humans and in animals (Martınez-Lopez *et al.*, 2009). In the case of the transmission of *B. burgdorferi s.l.*, a network must take into account that there are two separate types of node, hosts and vectors, and that transmission can only occur between two nodes belonging to different types (Bisanzio *et al.*, 2010). This is based on the assumption that transmission only occurs between vectors and hosts, and not among vectors (e.g. by transovarial passage of spirochaetes between successive generations of ticks) or among hosts. These types of networks can be defined as bipartite (or bipartite graphs).

A key parameter of networks is the degree or number of contacts that a node has with other nodes. In the case of the bipartite graph for *B. burgdorferi s.l.* transmission, the distribution of degree over the node family representing the host determines the aggregation of ticks feeding on hosts. In most networks, the distribution of the degrees of the nodes is highly aggregated. In fact, in general, there are individuals with many contacts and individuals with a limited number or no contact. The power law distribution is often the most appropriate to describe this characteristic, and Bisanzio *et al.* (2010) used it to model and simulate the transmission of *B. burgdorferi s.l.* and to account for individuals with very high tick loads (> 50 per host). The model confirmed, both by theoretical analysis and by numerical simulation, that aggregation of ticks among hosts favours transmission. Under these conditions, the threshold level of transmission was very low or absent, meaning that aggregation can cause the maintenance of the infection even with very low transmission levels of spirochaetes between ticks and hosts.

The key assumption of this approach is that the same individual hosts are particularly likely to be infested by nymphs and larvae. These individuals could therefore

serve as super-spreaders of *B. burgdorferi s.l.* Two scenarios of aggregation of ticks among vertebrate hosts are shown in Fig. 5. In both scenarios, A and B, larvae are aggregated among hosts. In scenario A, larvae and nymphs are aggregated on the same individuals, although at different times, and in this case, the positive effect of aggregation on the transmission of *B. burgdorferi s.l.* would be greatly enhanced. Conversely, in scenario B, the aggregation of larvae and nymphs may occur on different individuals with potentially negative effects on transmission (D. Bisanzio *et al.*, unpublished data).

Conclusions on the role of models in Lyme borreliosis ecological research

The few models for *B. burgdorferi s.l.* that have been considered in this review are useful to summarize current knowledge on the effects of multi-host systems for transmission and to gain insight into apparently contrasting effects, such as the balance between dilution of infection and vector augmentation by certain host species, that may shift in different ecological scenarios. Furthermore, the sensitivity of the simulated systems to changes in certain parameters allowed the identification of those key factors that determine whether biodiversity may dilute or amplify transmission of *B. burgdorferi s.l.* In this way, it is possible to understand why different conclusions can be drawn from field studies carried out at different locations. On the other hand, it is clearly hard to formulate general rules that are valid across locations, given the complexity of interactions among the various factors involved. As an example, attachment rates of different tick stages to different host species emerged as particularly important. Specifically, attachment rates of the nymphal stage to small rodents, and to other hosts characterized by varying reservoir competence, was identified as a key parameter in a wide range of multi-host systems and geographical areas.

Prediction of interactions of the effects of host population density and environmental factors (e.g. meteorological conditions) on dilution or amplification of transmission is difficult. For this reason, although models and threshold curves provide useful insights into complex transmission systems, numerical outputs should be considered with caution, because they might vary greatly under field conditions.

There is a long tradition in mathematical modelling of infectious diseases of humans and animals, but by far the largest body of literature has focused on viruses that can be transmitted directly among animals or humans
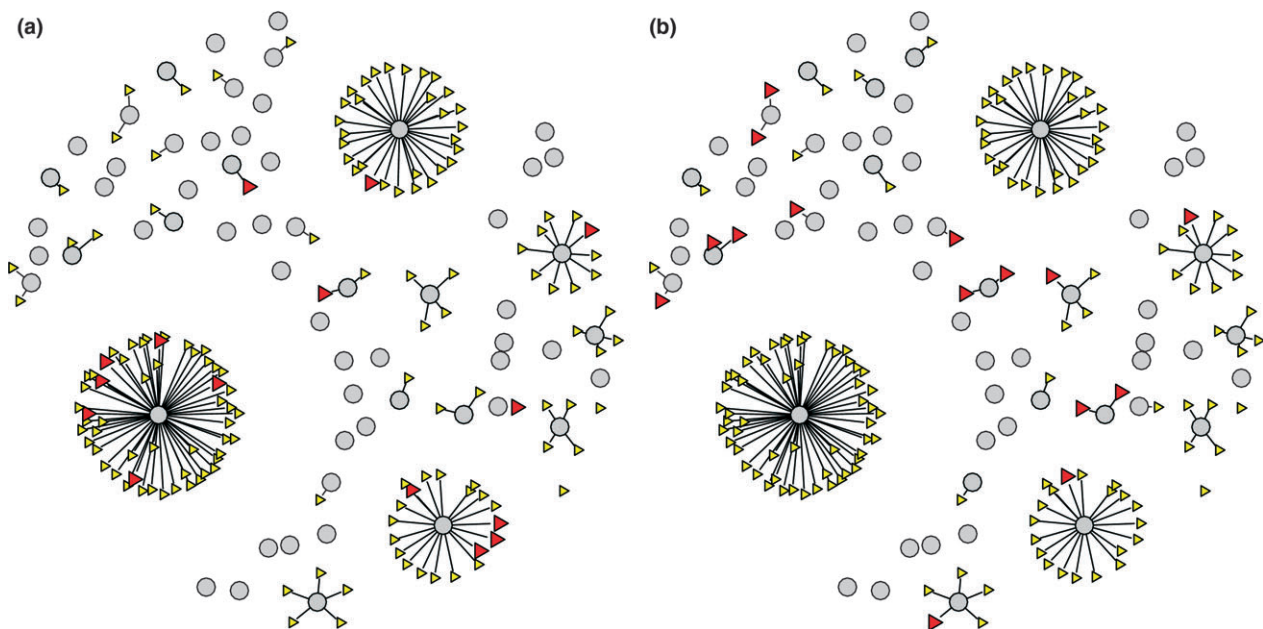

Fig. 5. Schematic representation of the aggregation of immature *Ixodes ricinus* among vertebrate hosts belonging to the same species (grey circles). (a) individuals infested by greatest numbers of larvae (small, yellow triangles) are the same individuals that were most likely to be infested by nymphs (large, red triangles). This may occur at different times, based on seasonal activity of immature tick stages. Under this scenario, transmission of *Borrelia burgdorferi sensu lato* from reservoir hosts to larvae is most efficient. (b) Aggregation of larvae and nymphs occur on different individual hosts. Under this scenario, most larvae, such as those feeding on the two hosts with greatest burdens, would not acquire the infection.

(Lloyd-Smith *et al.*, 2009). For these agents, standard model templates were developed for research and public health applications and are now part of epidemiology curricula. On the other hand, it is acknowledged that multi-host cycles and existing gaps in knowledge make Lyme borreliosis transmission difficult to model, and contrasting results may arise from different modelling approaches. For example, the possibility of modelling spirochaete transmission and tick and host population dynamics requires a choice in the level of generality of a model and increases its complexity.

The link between statistical models, that can be used to provide valid parameters estimates from field studies, and process models, where ecological processes are explored, is particularly important for ecological research. Statistical models are now available to deal with the complexity of the processes that generate the data, allowing account to be taken of important issues such as heterogeneous distribution of ticks among hosts using models for over-dispersed counts, for example negative binomial regression, or zero-inflated models, which are able to adjust for relatively large proportions of counts equal to zero (i.e. no ticks on animals). Network analysis can be used to further explore the effects of aggregation of ticks on hosts on *B. burgdorferi s.l.* transmission.

Molecular mechanisms in *B. burgdorferi s.l.* ecology

The ecological dynamics of *B. burgdorferi s.l.* are underpinned by molecular mechanisms that enable it to survive in both vector and host, and the spirochaete expresses certain genes to this end, depending on the environment to which it is exposed. Some of these gene products have direct interactions with tick proteins, whereas others bind to, or interact with, reservoir host proteins. Interestingly, *B. burgdorferi s.l.* enhances expression of certain tick genes that are beneficial for vector colonization and transmission from the vector to the host (Pal *et al.*, 2004; Ramamoorthi *et al.*, 2005). In this section, molecular basis of the interactions among *B. burgdorferi s.l.*, vectors and hosts is reviewed, presenting a brief overview of bacterial genetic features and molecular methods used to understand these interactions.

Genetic features of *B. burgdorferi s.l.* and molecular approaches

The *B. burgdorferi s.l.* genome is approximately 1.5×10^6 base pairs (Fraser *et al.*, 1997) and includes a single linear chromosome and 21 linear and circular plasmids, together containing 1780 genes (Barbour, 1988). A large portion of the genome encodes lipoproteins. Several

B. burgdorferi s.l. proteins have been identified that have interactions either with host or tick ligands and assist pathogen survival and transmission (Guo *et al.*, 1995; Ramamoorthi *et al.*, 2005; Neelakanta *et al.*, 2007).

Bacterial isolation (Barbour, 1984) is still considered the reference method for *B. burgdorferi s.l.* identification in diagnosis and in epidemiology, but molecular biology provides additional very useful tools. Ribosomal 16s RNA has been used for classification of the Spirochaetaceae family (Paster *et al.*, 1991), and restriction fragment length polymorphism protocols have been developed for easy genospecies identification (Postic *et al.*, 1994). Several methods have been developed for genospecies identification based on genetic sequencing, and a large number of genes are considered to be informative for phylogenetic studies. In fact, during the last few years, several investigations have been conducted to identify possible candidate gene sequences and to infer genetic relationships among genospecies. Genes encoding flagellin (Fukunaga *et al.*, 1996), outer surface proteins A (Grego *et al.*, 2007) and C (Alghaferi *et al.*, 2005), as well as the amplification of the variable intergenic spacer between repeated 23S (*rrl*) and 5S (*rrf*) ribosomal genes (Rijpkema *et al.*, 1995) are examples of DNA targets resulting from *B. burgdorferi s.l.* genome characterization. These single-gene protocols are very useful tools in the identification of the spirochaetes.

Several genes have been sequenced, and complete genomes and full plasmid sequences are now available on public databases. Multilocus sequence typing (MLST) currently represents one of the most accurate methods for the investigation of genetic characteristics of individuals, allowing a considerable amount of information to be obtained from gene sequences. Several recent studies have used sequence information of multiple loci to characterize *B. burgdorferi s.l.* (Bunikis *et al.*, 2004; Richter *et al.*, 2006; Postic *et al.*, 2007; Chu *et al.*, 2008). More recently, Margos *et al.* (2008) analysed the population structure of isolates, investigating a set of housekeeping genes and discussing the European origin of these agents. The presented data suggest that MLST can be used as a powerful means of studying the evolution and epidemiology of *B. burgdorferi s.l.* Moreover, MLST allows genotyping of *B. burgdorferi s.l.* samples and capturing evolutionary and geographical relationships among bacterial populations. The MLST approach has also been used to investigate the phylogeographical structure of *B. lusitaniae* in Portugal (Vitorino *et al.*, 2008). In this study, MLST highlighted population clustering at a fine spatial scale, showing the close genetic relationships among Portuguese and North African *B. lusitaniae* isolates. As suggested previously (Grego *et al.*, 2007), *B. lusitaniae* shows a geographical structure that includes two main lineages: MLST allows

the deeper exploration of spatial segregation of bacterial strains, reaching finer spatial scales and depicting the parallel evolution of isolated strains.

Host interactions

As discussed in the previous section, a wide range of vertebrate species is parasitized by *I. ricinus* ticks, but these host species vary in their reservoir competence for *B. burgdorferi s.l.*, and this underlies the complexity of transmission of the spirochaetes in multi-host systems. A vertebrate reservoir host must be able to (i) host vector ticks, (ii) acquire *B. burgdorferi s.l.* from infectious ticks, (iii) allow the agent to multiply and/or develop and persist in its body and (iv) transmit the agent back to subsequently feeding vector ticks (Kahl *et al.*, 2002). Transmission of *B. burgdorferi s.l.* among ticks through nonsystemic infection of the host, however, makes such a definition less rigid. A major issue is whether the concept of *B. burgdorferi s.l.* genospecies host specificity is ecologically relevant. This question has been notoriously difficult to address, partly for technical reasons, but mainly owing to the complexity of the transmission systems. In their review of 2002, Kurtenbach *et al.* described in detail the pathways of *B. burgdorferi s.l.* transmission from ticks to hosts and vice versa (Kurtenbach *et al.*, 2002a). Again in 2002, Kurtenbach *et al.* proposed a detailed and comprehensive explanation of the role of the host complement system in host infection (Kurtenbach *et al.*, 2002b). Certain vertebrates are considered to be barriers for specific genospecies of spirochaetes. These hosts are incompetent reservoir hosts but feed ticks and therefore may contribute to the transmission of *B. burgdorferi s.l.* through maintenance and augmentation of the vector population. To understand the key factors in the *B. burgdorferi s.l.* life cycle, we need to examine in depth the molecular mechanisms that drive interactions in this complex system.

Outer surface proteins (Osps) and salivary proteins (Salps)

While infected ticks engorge, the antigenic composition of *B. burgdorferi s.l.* changes drastically. *Borrelia burgdorferi s.l.* genes up-regulated in engorging *Ixodes* nymphs include those encoding putative lipoproteins and periplasmic proteins (Narasimhan *et al.*, 2002). Surface proteins (Osp) belong to the most important protein antigens of *B. burgdorferi s.l.*, playing an essential role in the pathogenesis. The first strategy of *B. burgdorferi s.l.*, to avoid the destructive activity of the host immune system, is represented by the change in the synthesis of surface proteins and adaptation to different hosts.

The biological function of OspA, a *B. burgdorferi s.l.* protein produced by the spirochaete while it resides in the tick gut (de Silva & Fikrig, 1997) but which is down-regulated during tick engorgement, has been established. A strain of *B. burgdorferi s.s.* deficient for OspA and OspB was able to infect mice and cause arthritis (Yang *et al.*, 2004), but could not colonize the tick gut (Pal *et al.*, 2000). This indicates that OspA and/or OspB have an important role in persistence of the spirochaete within the vector (Pal *et al.*, 2004).

Selective and temporal expression of OspA and OspB in ticks suggests that these two proteins may be engaged in early colonization and survival of the spirochaete in the tick. OspA mediates in the adherence of spirochaetes to the tick's gut through binding TROSPA proteins (Pal *et al.*, 2004). OspA has also been found to stick to itself and thereby may further facilitate spirochaete–spirochaete adherence and interaction (Pal *et al.*, 2000). OspB, which shares a promoter with OspA (Howe *et al.*, 1986), also seems necessary for *B. burgdorferi s.l.* adherence to the tick midgut, although the putative midgut receptor has not been identified (Neelakanta *et al.*, 2007). OspA and B probably help *B. burgdorferi s.l.* to remain attached to the midgut, rather than being actively removed or destroyed by the tick, during feeding and/or moulting (Tsao, 2009).

During the tick blood meal, *B. burgdorferi s.l.* modifies the expression of several genes. The most evident shift is the down-regulation of *ospA* and up-regulation of *ospC* (Schwan & Piesman, 2000). This shift in the expression of this pair of genes has become a paradigm for the differential expression of suites of genes associated with different steps in the natural transmission cycle of *B. burgdorferi s.l.* between the tick and vertebrate hosts. In the case of OspC, an abundantly expressed Osp, this shift reduces the efficiency of the vertebrate host adaptive immune response in eliminating spirochaetes in the tick midgut. Because spirochaetes down-regulate OspC expression in the tick midgut, host anti-OspC antibodies ingested by an infected tick cannot prevent transmission from the tick to the vertebrate (Tsao, 2009).

Borrelia burgdorferi s.l. enhances expression of certain tick genes, among which is *salp15*, a gene encoding for a 15 kDa feeding-induced salivary gland protein. A study in the United States showed that Salp15 from *I. scapularis* interacts with *B. burgdorferi s.l.* by binding to OspC, thus protecting the spirochaete from antibody-mediated killing in the host and facilitating successful early infection (Ramamoorthi *et al.*, 2005). Syringe infection of naïve mice with *B. burgdorferi s.l.* and recombinant Salp15 resulted in significantly higher spirochaete numbers compared to infection with spirochaetes alone. Moreover, RNA interference-mediated repression of Salp15 in *I. scapularis* ticks drastically reduced the capacity of these

ticks to transmit spirochaetes to mice (Ramamoorthi *et al.*, 2005). In nature, increased Salp15 levels could also be beneficial for ticks that might use Salp15's immuno-suppressive capacities to avoid rejection by the host and to engorge more effectively.

Interestingly, Salp15 homologues were recently also identified in the European vector, *I. ricinus* (Hovius *et al.*, 2007). Tick saliva from *I. ricinus* was shown to assist *B. burgdorferi s.l.* in evading host skin innate immune responses by suppressing the production of antimicrobial peptides induced by *B. burgdorferi s.l.* (Marchal *et al.*, 2009).

Erp and CRASP proteins that bind H, FHL1 or C3 to repress complement cascade

When *B. burgdorferi s.l.* infects a host, the first obstacle is the immune system. A crucial line of defence in the innate immune response against invading microorganisms is the complement cascade, consisting of the classical, lectin and alternative pathways. Many bacteria have evolved mechanisms to inhibit complement-mediated killing by binding to host plasma factor H or factor H-like (FHL) protein resulting in factor I-mediated degradation of C3b. It is now well recognized that many pathogenic bacteria, including *B. burgdorferi s.l.*, bind FH/FHL-1 on their cell surface to evade complement-mediated destruction during infection (Zipfel *et al.*, 2002; Skotarczak, 2009). *Borrelia burgdorferi s.l.* utilizes complement regulator-acquiring surface proteins (CRASP) (Kraiczy *et al.*, 2001) and Osp E/F-related proteins (Erp) (Alitalo *et al.*, 2001) to bind factor H or FHL protein and consequently inhibit complement-mediated activity.

Currently, five CRASP proteins binding FH and FHL-1 (CRASP-1 and CRASP-2), or only factor H (CRASP-3, CRASP-4, CRASP-5 and Erp proteins) have been identified (Alitalo *et al.*, 2002). Among CRASP proteins, the protein CRASP-1 in *B. burgdorferi s.l.* binds the main factors FH and FHL-1, providing immunity to complement in *in vitro* culture (Kraiczy *et al.*, 2004). The latest studies have shown that inactivation of the gene encoding CRASP-1 in *B. burgdorferi s.l.* gives results in the serum-sensitive phenotype, and addition of mutated strain with CRASP-1 restores the resistance for lysis initiated by the complement (Brooks *et al.*, 2005). These data suggest that CRASP-1 facilitates immunoevasion and survival of spirochaetes in humans (Rossmann *et al.*, 2006; Hallström *et al.*, 2010), but the expression of CRASP-1 during the infection in humans is still under discussion (Von Lackum *et al.*, 2005).

On the other hand, Erp lipoproteins are from the group of specific proteins synthesized by *B. burgdorferi s.l.* during the infection of mammals, and their function is thought to be the same as that of other proteins from this group. It consists of binding the protein factor H from the host's serum during the alternative way of complement activation (Alitalo *et al.*, 2002; Stevenson *et al.*, 2002; Kraiczy *et al.*, 2004).

Molecular probes for identification of tick and reservoir hosts

Several DNA probes have been developed to investigate the role of host species involved in the circulation of *B. burgdorferi s.l.* in nature. Host DNA in the remnants of larval blood meals can be detected in the gut of *I. ricinus* nymphs for several months after moulting and similarly in the gut of adults that moult from nymphs. Thus, identification of host blood in a tick that has, in parallel, been found to contain DNA of a particular pathogen makes it possible to draw conclusions about the reservoir status of the detected host. The first attempt to use this technology targeted the mitochondrial *cytochrome b* gene using a PCR followed by reverse line blot. It was possible to detect individual host species but generic primers for the PCR component did not cover a sufficient range of potential hosts (Kirstein & Gray, 1996). Targeting the 18S rRNA gene was more satisfactory for detection of blood from taxonomic groups of animals, but because this gene is not found in the mitochondria, there was a consequent loss of sensitivity for the detection of mammal blood compared with that from birds (Pichon *et al.*, 2003, 2005).

The most recent and promising studies have targeted the 12S rRNA gene (Humair *et al.*, 2007; Morán Cadenas *et al.*, 2007a). Host DNA was detected in more than 40% of samples, and hosts were successfully identified at the genus and species level in 72% of these (Morán Cadenas *et al.*, 2007a). Host identification success may vary locally and temporally. In fact, Morán Cadenas *et al.* (2007a) reported that identification in one site was most successful in spring (93% in May) and autumn (73% in October), whereas in the other site, identification rates remained low (between 20% and 40%) but increased in autumn reaching 68% in October and November. These results raise questions about local *I. ricinus* population seasonal dynamics, which may vary according to very specific climatic conditions.

The blood meal analysis method coupled to the detection of *B. burgdorferi s.l.* in the same ticks permitted further examination of the host specificity of the spirochaetes, confirming the association between specific genospecies and birds and rodents (Gern & Humair, 2002). However, absence of a strict association between *B. burgdorferi s.l.* genospecies and particular hosts was also observed, supporting reports that hedgehogs

(*E. europaeus*), badgers (*M. meles*) and roe deer (*Capreolus capreolus*) may harbour the same genospecies (Gern *et al.*, 1997; Pichon *et al.*, 2000; Gern & Sell, 2009). Other studies on blood meal analysis targeting the 18S rRNA gene have also shown both strict and loose associations between *Borrelia* genospecies and hosts (Pichon *et al.*, 2003, 2005, 2006; Estrada-Peña *et al.*, 2005). The simultaneous use of the two approaches allowed confirmation of the fox (*V. vulpes*) as a reservoir host (Morán Cadenas *et al.*, 2007a), already reported by Kahl & Geue (1998) and Liebisch *et al.*(1998) and also permitted exploration of the reservoir status of hosts that are difficult to study such as wild boar (*Sus scrofa*) (Estrada-Peña *et al.*, 2005).

Conclusions on molecular mechanisms in Lyme borreliosis ecology

Considering the large number of genes involved in pathogen–tick-host interactions, it is very difficult to understand exactly how *B. burgdorferi s.l.* infection is modulated by different host species. The immunological response against spirochaete and tick antigenic proteins is adaptive and the bacteria must find a way to 'escape' to survive. Genetic heterogeneity among and within *Borrelia* genospecies can be a useful means to adapt antigenic patterns to specific host responses and can enable *B. burgdorferi s.l.* to infect different hosts.

Genetic features can describe and explain how *Borrelia* genospecies interact with hosts, and increased knowledge of heterogeneity in nucleotides and, consequently, in amino acid sequences is fundamental to a better understanding of the relationships among all the participants involved in pathogen–vector–host systems.

Genetic characterization helps in the understanding of how *Borrelia* genospecies can adapt their life cycles to specific hosts but it is equally important to understand whether other factors play a role in the distribution and diffusion of infections. When viewing this problem at a larger scale, infection at the population level, it is clearly important to investigate genetic differences among bacterial strains at the geographical level. Scientists need to understand the differences among *B. burgdorferi s.l.* strains occurring in different areas, where, for example, vector and host compositions are different. Moreover, habitat and climatic features are very important for the maintenance of vectors and hosts, so that focusing on genetic characteristics at the population level can help explain relationships among the participants in the *B. burgdorferi s.l.* transmission cycle. Heterogeneity among and within *B. burgdorferi s.l.* genospecies has been investigated in relation to host preferences, and the bacterial population structure was the main goal in several recent studies, which provided very detailed results on phylogenetic relationships among *B. burgdorferi s.l.* isolates (Grego *et al.*, 2007; Vennestrøm *et al.*, 2008; Comstedt *et al.*, 2009; Margos *et al.*, 2009). In the future, information will also be required on the genetic heterogeneity of tick populations because not only may different tick populations show variations in pathogen/tick interactions, but variations in vector population dynamics and seasonal activity may also have a genetic basis, which would affect the eco-epidemiology of Lyme borreliosis. Population genetic studies on the seabird tick *Ixodes uriae* have shown that host-specific tick races occur in both the Northern and the Southern Hemispheres (Kempf *et al.*, 2009), and Gomez-Diaz *et al.* (2011) demonstrated distinct differences between these tick races in *B. garinii* infection intensity. Similar studies on *I. ricinus* are currently in progress (Kempf *et al.*, 2011).

Together with the increasing amount of genetic data, several computational approaches have been developed and are used to describe genetic relationships among isolates. Briefly, *a priori* and *a posteriori* statistical methods, based on models of molecular evolution, are very frequently used to produce more robust results on genetic population structure; maximum likelihood (Felsenstein, 1973) and bayesian approaches (Geyer, 1991) are the most utilized methods for the study of phylogeny. These are useful tools for eco-epidemiological investigations as well as for isolate characterization. The integration of such quantitative approaches with molecular methods for the investigation of pathogen and vector genetic heterogeneity will help to analyse vector-borne disease epidemiology in a climate change context, one of the most pressing environmental concerns of today.

Effects of climate change on the ecology of *B. burgdorferi s.l.*

(This section summarizes relevant parts of a previous review on this topic by Gray *et al.*, 2009).

The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Christensen *et al.*, 2007) reported that in northern temperate Europe, temperature increases of 1.5–2.5 °C may occur over the next few decades as a result of global warming. Because the main European vector of *B. burgdorferi s.l.*, *I. ricinus,* spends most of its life cycle in the external environment rather than on its hosts, such a climate change is likely to affect tick survival, development and reproduction, while climate effects on the numbers, migration patterns and diversity of hosts will impact tick abundance and distribution. The incidence and distribution of Lyme borreliosis is also certain to be affected through the complex interactions of tick biology and that of tick hosts with

climate variables. However, despite these theoretical considerations, there are as yet few data showing unequivocal influences of climate change on either ticks or Lyme borreliosis. Possible effects are considered here in relation to tick development and survival in the external environment, acquisition of hosts for the maintenance of tick and spirochaete populations, and the occurrence of Lyme borreliosis.

Effects on tick development, survival and host-seeking activity (questing)

The microclimatic requirements of *I. ricinus* for successful development and survival include a relative humidity of at least 80% to avoid fatal desiccation of the free-living stages, so that *I. ricinus* is restricted to areas of moderate to high rainfall where there is a good cover of vegetation, and where the soil surface remains humid through the driest times of the year (Gray, 1998). In areas where lowered summer precipitation coincides with raised summer temperatures, the survival, activity and distribution of *I. ricinus* are likely to be reduced because of the restriction of suitably moist summer habitat. Such climate changes will also affect the nature of the vegetation cover, in some cases adversely affecting survival rates of free-living stages by drying out the microhabitat, but on the other hand favouring survival, for example by causing the replacement of Norway spruce (*Picea abies*) by beech (*Fagus sylvatica*) (Kölling, 2008), the fallen leaves of which provide a suitable microclimate for ticks. Climate change may also affect the availability of suitable tick hosts by influencing faunal abundance and diversity (Fischlin *et al.*, 2007). This would affect the circulation of spirochaetes between reservoir hosts and ticks, and also impact tick population growth and distribution. The most important hosts for maintenance of tick populations in Europe are cervids, especially roe deer (*C. capreolus*), and a warmer climate in northern regions or at high altitudes would result in better winter survival and earlier birth dates thus increasing overall deer abundance. Higher temperatures would also increase plant growth so that habitats would support denser deer populations. Conversely, in warm southern regions an increase in temperatures may result in lowered deer abundance.

There is also evidence that high summer temperatures (> 30 °C) can change seasonal host-seeking activity patterns by altering the proportion of the tick population that are exposed to regulatory mechanisms such as diapause. A study in Ireland suggested that hot summers may cause ticks to become more abundant in autumn and early winter, primarily through diapause effects, resulting in a shift in activity from spring to autumn for a large proportion of the tick population (Gray, 2008). In

this way, the host-seeking activity of *I. ricinus* could change to a more southern European pattern (Estrada-Peña *et al.*, 2004) showing increased winter activity, especially if winters become warmer so that available ticks can become active.

It is well known that ambient temperatures affect tick host-seeking activity, with a lower limit (mean maximum air temperature) of 7 °C (MacLeod, 1936; Gray, 1984; Perret *et al.*, 2000). Increased tick activity was demonstrated in the unusually mild winter of 2006/2007 in eastern Germany, with concomitant early cases of TBE, a viral tick-borne disease (Dautel *et al.*, 2008). The upper temperature limit for host-seeking activity is not clear because it depends to a large extent on prevailing saturation deficits. Data from Switzerland show that tick activity may decrease in hot summers (Perret *et al.*, 2000), an effect probably due to both tick sequestering behaviour and also poor survival resulting from raised saturation deficits (Perret *et al.*, 2004). However, Irish data (Gray, 1984) showed that all active stages of *I. ricinus* will quest throughout hot dry weather at temperatures up to 35 °C as long as appropriate vegetation cover is present to provide opportunities for rehydration. The same situation appears to obtain for *I. scapularis* immature stages in the USA (Schulze *et al.*, 1986). Interestingly, in the southern distribution of *I. ricinus*, in Tunisia, it was observed that larvae and nymphs have their peak of questing activity during some of the warmest months of the year (May, June and July) (Dsouli *et al.*, 2006).

Effects on tick distribution

The northern limit of the distribution of *I. ricinus* is determined by low temperatures. Although acclimatized unfed individuals can survive temperatures as low as −18.9 °C for 24 h, only −10 °C proves lethal over a thirty day period (Knülle & Dautel, 1997). While developing, *I. ricinus* is even more sensitive to low temperatures and little or no development takes place between 7 and 10 °C (Campbell, 1948; Kahl, 1989), so at high latitudes summer temperatures may be too low to complete development before the onset of winter. There is evidence from Sweden that the northern distribution limit of *I. ricinus* has shifted northwards since the climate started to change noticeably in the late 1980s (Tälleklint & Jaenson, 1998) (Fig. 6). This shift in latitude distribution appears to be related to changes over several seasons in the number of degree-days with temperatures vital for tick survival, activity and development (Lindgren *et al.*, 2000).

Several studies have reported changes in the altitudinal distribution of *I. ricinus,* also apparently driven by a
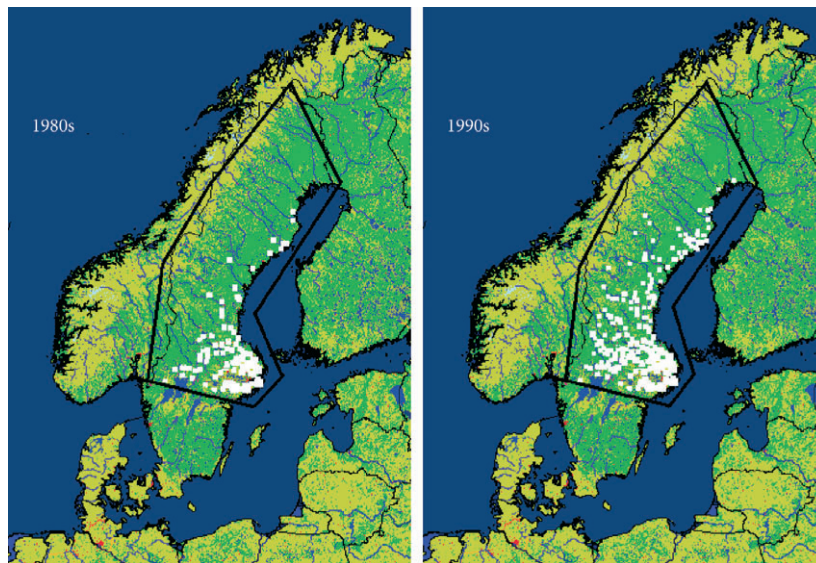
Fig. 6. Changes in the distribution of *Ixodes ricinus* (white dots) in Sweden indicated by comparison of pre-1980 data with 1994–1995 data (reproduced from Lindgren *et al.*, 2000; with permission from *Environmental Health Perspectives*).

warming climate. In studies conducted in 1957 and 1979–1980 in the mountainous regions of the Czech Republic, ticks were found up to 700 m above sea level (m.s.l.), but in the last 10 years have been found up to 1300 m.s.l. (Daniel *et al.*, 2004, 2009; Materna *et al.*, 2005). In Switzerland, in two studies along an altitudinal gradient in 1999–2001 (Jouda *et al.*, 2004b) and 2003–2005 (Morán Cadenas *et al.*, 2007b), substantial differences in phenology and density of ticks were observed at low and high altitudes. At high elevations, ticks displayed peaks of questing activity and were significantly more abundant during the second study period. One proposed explanation was rising temperatures recorded during summer at altitudes, reaching values that were similar to those registered at the lowest altitude during the first study (Morán Cadenas *et al.*, 2007b). At the lowest altitude, high temperatures and moisture stress had a negative effect on tick abundance.

Modelling the effects of climate on ticks

A study on the changes in climate suitability for *I. ricinus* in the western Palaearctic has been carried out using deme-derived models (demes being local populations of a species sharing the same gene pool) based on different populations recognized in the Palaearctic (Estrada-Peña & Venzal, 2006). A long (1900–1999) series of climate data at coarse resolution (10 min of arc) were used to examine trends in climate and to estimate sustained variations in climate suitability for *I. ricinus*. While some areas showed a deterministic (i.e. continu-

ous) tendency towards increasing or decreasing suitability for the tick, others showed unambiguous cycles of climate suitability, termed areas of 'random walk'. In these, populations of the tick may undergo periodical variations in their geographical range as a consequence of cyclic changes in climate. This analysis suggests that while climate suitability for *I. ricinus* has not changed over Europe as a whole during the 100-year study period, it increased or decreased in specific geographical regions. These changes are not recent and are associated with yearly and summer rainfall patterns rather than with temperature. The reported increased abundance of *I. ricinus* in parts of Europe, for example in Sweden (Tälleklint & Jaenson, 1998), coincides geographically with the regions where a recent increase in climate suitability has been detected, within zones having a marked 'random walk' tendency. No single variable was consistently associated with changes in climate suitability across sites where 'random walk' was detected. The absence of a single regulatory variable seems to be connected with the different climate niche experienced by the tick populations in their distribution area. Thus, rainfall and temperature have different regulatory abilities according to the portion of the tick's climate envelope represented in a given area.

Climate data based on climate change predictions, together with phylogenetic data on the potential response of *I. ricinus* populations to climate parameters, could be used in such models to indicate the possible impact of climate change on the future distribution of ticks over large areas.

Effects on the distribution and incidence of Lyme borreliosis

Lyme borreliosis occurs at a relatively high incidence for a zoonotic disease, ranging from 155 per 100 000 in Slovenia to 0.6 per 100 000 in Ireland (Lindgren & Jaenson, 2006). However, unlike TBE, which is notifiable in many countries, the reliability of Lyme borreliosis incidence data is uncertain because of diagnostic problems and limited or nil reporting. Nevertheless, in some regions it has proved possible to relate disease incidence to climate, for example, a positive association with mild winters and warm, humid summers has been reported in southern Sweden (Bennet *et al.*, 2006). The mechanisms responsible for this are not clear but mild winters may extend the tick activity season, increasing host acquisition success towards the end of the year, thus resulting in greater numbers of infected ticks becoming available in the following year. Fine weather in summer is likely to result in an increase in outdoor activities generally, resulting in increased exposure to tick bites. Tick bite risk is further enhanced when humidity is high in warm weather, because this favours tick activity and is also associated with mushroom gathering (Bennet *et al.*, 2006). The occurrence of *B. burgdorferi s.l.* in ticks can be regarded as a proxy for the presence of Lyme borreliosis in an area and Daniel *et al.* (2009) reported that ticks that have recently colonized high-altitude habitats were infected with *B. burgdorferi s.l.*, including the pathogenic genospecies, *B. afzelii* and *B. garinii*. The presence of *B. burgdorferi s.l.* was not investigated in *I. ricinus* collected in the northward extension of their range in Sweden (Lindgren *et al.*, 2000), but Gustafson *et al.* (1995) reported that all Swedish *I. ricinus* populations investigated up to that date have been infected, and Jaenson *et al.* (2009) asserted that infected ticks are present in every Swedish province.

There is now considerable evidence that the incidence of Lyme borreliosis throughout Europe has increased in recent decades (Hofhuis *et al.*, 2006; Smith & Takkinen, 2006; Fülöp & Poggensee, 2008; Heyman *et al.*, 2010), but the causes of this increase is likely to be multifactorial and the role of climate change is far from clear. A detailed study of the records of TBE incidence over the last three decades in the Baltic States showed that although disease incidence rose dramatically, it was impossible to identify climate change as a direct factor (Sumilo *et al.*, 2007; Randolph *et al.*, 2008). The same situation is likely to apply to the Europe-wide increase in Lyme borreliosis incidence, especially because accurate figures are so much harder to obtain than incidence data for TBE.

An intriguing recent study in the United States suggested that climate change may alter the relative proportions of *B. burgdorferi s.s.* genotypes in a region by influencing the timing of activity of larval and nymphal *I. scapularis* (Gatewood *et al.*, 2009). As some genotypes do not persist in their reservoir hosts for more than a few weeks, it follows that they have a higher chance of being acquired by larvae if nymphs feed at the same time of year. Gatewood *et al.* (2009) showed that the magnitude of the difference between summer and winter temperature maximums was positively associated with the degree of seasonal synchrony of the tick immature stages, and that one particular genotype had a higher prevalence than others in areas where seasonal synchrony was low. No such studies have been performed in Europe, but the timing of larval and nymphal *I. ricinus* activity also varies in different climatic zones in Europe, and the degree of seasonal synchrony, apparently determined by relatively rapid warming in spring, has been shown to underlie the endemicity of TBE (Randolph, 2009). If this climate effect on tick questing activity is true, one might expect climate change to influence the efficiency of transmission of different *B. burgdorferi s.l.* genotypes depending on their relative persistence in reservoir hosts.

A related field of research that has been poorly researched so far is the effect of climatic stress on infected ticks. It was recently observed that the survival of *I. ricinus* nymphs and adults under challenging climatic conditions was significantly enhanced by infection with *B. burgdorferi s.l.* (Herrmann & Gern, 2010). These authors reported that infection by *B. burgdorferi s.l.*, and more specifically infection by *B. afzelii*, confers survival advantages on *I. ricinus* under stressful thermo-hygrometric conditions. This may be related to field observations in Switzerland where Morán Cadenas *et al.* (2007a) reported a higher density of *Borrelia*-infected ticks during 2003–2005, a period showing higher summer temperature, than during 1999–2001, in the same area (Jouda *et al.*, 2004b). Further analysis of the whole situation, at various levels, could help explain the present distribution of *B. burgdorferi s.l.* genospecies in Europe and also predict future distributions resulting from climate change.

Conclusions on climate change effects on Lyme borreliosis ecology

There seems little doubt that global warming is already affecting the life cycles of ticks and their transmission of diseases, but the complexity of the interacting factors that determine the timing and degree of tick activity and disease transmission makes it impossible at present to predict likely Lyme borreliosis incidence in future climate change scenarios. Data from long-term studies on tick biology, distribution and abundance, host abundance and distribution, Lyme borreliosis incidence and relevant vegetation biology, specifically in relation to climate change, are required to develop models that, in addition to

determining the probability of climate conditions suitable for the vectors of Lyme borreliosis, also take account of dynamic biological processes.

General conclusions

Ecological research on *B. burgdorferi s.l.* is based largely on the principles of population ecology applied to population dynamics of hosts and vectors, and to the distribution of pathogens and vectors among hosts. In this context, appropriate field data collection and statistical and mathematical models play a key role. However, in addition to the quantitative relationships among its components, the study of ecological mechanisms underlying the transmission of *B. burgdorferi s.l.* must also include the molecular processes that have a central role at the interfaces between hosts, ticks and spirochaetes. Integration between quantitative and molecular approaches is required, not only in the final phase of data analysis, but especially when designing the collection of field data. As an example, molecular tools can be instrumental in evaluation of critical parameters that are identified by statistical and mathematical analysis, such as transmission. The interactions between climate change and the other factors affecting the ecological dynamics of *B. burgdorferi s.l.* can only be fully investigated using this integrated approach.

In Europe, large amounts of data on the ecology of *B. burgdorferi s.l.* have been produced by field studies and subsequent laboratory analysis. Nevertheless, conclusions are difficult to formulate on key ecological issues, such as the role of host species or of general biodiversity in the transmission cycle. This is due not only to the natural variability and complexity of the system, but also to extreme heterogeneity in sampling design and data analysis. Although complete harmonization is unrealistic in research, a careful definition of study design and the consistent application of appropriate tools for analysis, taking into account data type and structure, would improve comparability across studies and their general usefulness. Ecological research should generate the knowledge and information to provide the scientific basis for the protection of public health. Animal hosts of vector-borne agents, for example, may not only be the targets of infection-control measures, but can also be profitably used in data collection for surveillance and risk assessment. The identification of the role of particular hosts species in transmission cycles (together with their responses to the infection) is particularly important for risk assessment and risk management, especially if indicator species for the disease in question can be determined. Modelling the population dynamics of indicator host species in response to climate change would make a major contribution to the assessment of future climate effects on ticks and tick-borne pathogens.

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