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Title

What do we still need to know about *Ixodes ricinus*?

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Abstract

In spite of many decades of intensive research on *Ixodes ricinus*, the castor bean tick of Europe, several important aspects of its basic biology remain elusive, such as the factors determining seasonal development, tick abundance and host specificity, and the importance of water management. Additionally, there are more recent questions about the geographical diversity of tick genotypes and phenotypes, the role of migratory birds in the ecoepidemiology of *I. ricinus*, the importance of protective immune responses against *I. ricinus*, particularly in the context of vaccination, and the role of the microbiome in pathogen transmission. Without more detailed knowledge of these issues, it is difficult to assess the likely effects of changes in climate and biodiversity on tick distribution and activity, to predict potential risks arising from new and established tick populations and *I. ricinus*-borne pathogens, and to improve prevention and control measures. This review aims to discuss the most important outstanding questions against the backdrop of the current state of knowledge of this important tick species.

Keywords: *Ixodes ricinus*, Water balance, Biodiversity, Migratory birds, Genetic variation, Microbiome

Introduction

Ixodes ricinus, the most abundant tick in Europe, has been the subject of intensive research for more than 90 years, resulting in at least 4,000 publications − more than for any other tick species. Four distinct periods of research on this species can be distinguished. The classic early studies were conducted from the 1930s to the 1950s (reviewed by Arthur, 1962) and mainly concerned its role as a parasite of sheep. A second phase of intensive research was prompted by the emergence of tick-borne encephalitis in central Europe and the incrimination of *I. ricinus* as the vector of the causative virus (Rampas and Gallia, 1949). A major outcome was the demonstration that, unlike in the British studies, which focused on open hill land, the main tick habitat in these regions is deciduous and mixed woodland, as it is in most of the geographical range of *I. ricinus*. Perhaps the most influential research on tick biology in this phase concerned the role of photoperiod in the induction of diapause, which has emerged as the fundamental regulating mechanism of *I. ricinus* seasonal activity. The third major phase of *I. ricinus* research commenced with its identification in Europe as the vector of *Borrelia burgdorferi* sensu lato (Burgdorfer et al., 1983), the cause of the zoonosis, Lyme borreliosis. This, coupled with major advances in the molecular detection of microbial pathogens, has resulted in increased research on other known *I. ricinus*-transmitted pathogens. In the fourth and most recent research phase, new data on established pathogens have been collected and new pathogens discovered.

Many reviews of this body of research have been published. However, much of the information in such reviews is now very well known, and it is difficult to avoid restating facts and figures that have appeared multiple times, sometimes with repeated inaccuracies. Current research tends to emphasise the growing number of pathogens that *I. ricinus* carries and transmits and little attention is paid to some of the basic biological questions that have persisted for many decades. This review seeks to address areas of *I. ricinus* biology that are relevant to current and future issues, but have long been neglected or are still unresolved. Unfortunately, it has not been possible to include every important topic in detail, for example, diapause, which has recently been reviewed in depth, and the external factors that trigger the critical drop-off decision of the fully engorged *I. ricinus* in each of its life stages. However, exciting new avenues of research resulting from the development of new technologies, many of which are highly relevant to disease risk assessment and the implementation of control measures, are considered. Our overall objective is to contribute to a greater understanding of the necessary direction of future research on *I. ricinus*.

Host specificity

Ixodes ricinus shows a remarkably low degree of host specificity, illustrated by the fact that it has been recorded from more than 300 terrestrial vertebrate species (Gern et al., 2002). However, little research has been conducted to determine the biological basis for this relative lack of host specificity. Ecological factors, determining the possibility of contact between tick and host populations, are straight-forward and well understood. However, where *I. ricinus* has access to a variety of host species, the basis of host selection and the physiological adaptations that enable *I. ricinus* to feed successfully are complex and not fully elucidated.

Tick behavioural factors come into play during questing, resulting in host-seeking ticks responding to the presence of potential hosts. For example, there is some evidence that *I. ricinus* is attracted to sheep (Lees, 1948) and rodent odours (van Duijvendijk et al., 2017). However, *I. ricinus* engages in an ambush strategy and responds primarily to non-specific stimuli, such as carbon dioxide gradients, vibration, sudden shadow and local temperature increases (Lees, 1948), so that contact with a particular host species is probably mainly a matter of chance and behavioural factors may be more important for host specificity during the process of tick attachment to the skin of the host. Both Totze (1933) and Lees (1948) showed that taste and smell are involved by removing the palpatory organs on the palps and/or Haller's organs on the first pair of legs. Attachment occurred if one or the other was left intact, but not if both were removed. Observations on the reattachment of dislodged ticks suggest that many tick species lose all discrimination after the first attachment process is complete (Hoogstraal and Aeschlimann, 1982). To date no studies of attachment preferences to different host species have been conducted in *I. ricinus*, though the extensive host record suggests that this tick species is not very discriminatory. However, the number of truly important hosts of the three feeding stages of *I. ricinus* is probably more limited than the records suggest because feeding success is not usually taken into account. Examples of recorded hosts of adults, but with no indication of feeding success, include pheasant (*Phasianus colchicus*) and red grouse (*Lagopus lagopus scotica*) (Milne, 1949). Amongst mammals, wild rabbits (*Oryctolagus cuniculi*) and wild boar (*Sus scrofa*) are frequently listed as hosts, but the data suggest that these species contribute little to the maintenance of *I. ricinus* (J.S. Gray unpubl. observ.; Ruiz-Fons et al., 2006; Adamska, 2008; Pacilly et al., 2014; Takumi et al., 2019). Mustelids apparently contribute less than 1% to tick populations (Hofmeester et al., 2018). Foxes are poor hosts for both larvae and nymphs, although adult females can feed to repletion under laboratory conditions (Kahl and Geue, 1998). Deer, especially roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*), both native to northern and central Europe, are clearly natural hosts of *I. ricinus*, but sheep are not (despite the common English name of sheep tick for *I. ricinus*), having been originally introduced from the Middle East. Sheep and other domestic livestock are capable of maintaining populations of *I. ricinus* in the absence of other hosts (Gray, 1998), but sheep can develop strong resistance to this tick species (Abdul-Amir and Gray, 1987), with a histopathology that closely resembles that of rabbits, another 'unnatural' host (Brossard and Fivaz, 1982). So far no methodical comparative studies on the subsequent performance (engorgement, development, egg laying) of *I. ricinus* after attachment to different host species have been conducted.

The three feeding *I. ricinus* life stages show a certain level of discrimination even among the most frequently and intensely infested host species. For example, it is commonly stated that larvae feed primarily on small mammals, nymphs on reptiles, medium-sized mammals and birds, and adult females on medium-sized and large mammals (Hofmeester et al., 2016). However, the role of small mammals as the main hosts of larvae may be habitat and host-density dependent, since birds are commonly infested, and sheep (Gray, 1984), roe deer (Tälleklint and Jaenson, 1997) and red deer (Gilbert et al., 2012) can also feed very large numbers of larvae, mainly on the face and feet, as well as nymphs mainly on the ears (Handeland et al., 2013). The lack of recognition of the important role of deer in feeding immature tick stages may be because most investigators rely on actual counts in situ and few have counted immature stages by allowing them to detach from host carcasses and fall into trays of water (Tälleklint and Jaenson, 1997). The basis for the feeding predilections of the different stages of *I. ricinus* on various hosts is still uncertain.

Recording numbers of ticks on trapped or hunted animals remains the main method of determining host specificity, despite the difficulties in estimating feeding success. An alternative approach is to identify, by PCR and sequence analysis, the host origin of the residual blood from the previous meal of questing ticks. The first studies targeted the *cytochrome b* (Kirstein and Gray, 1996), 18S rRNA (Pichon et al., 2003) and 12S rRNA genes (Humair et al., 2007), and some success has been achieved in identifying hosts in field studies using these genes (Gray et al., 1999; Pichon et al., 2005; Morán Cadenas et al., 2007; Honig et al., 2017). However, declining sensitivity is a major limitation because of host DNA degradation over the long periods (more than 6 months) that can elapse between larval and nymphal engorgement and the appearance of the next questing stages on the vegetation, usually the following year (Gray et al., 2016). Furthermore, reliability and cost effectiveness are problematic and despite various refinements (Gariepy et al., 2012; Collini et al., 2015) and the application of alternative technologies such as proteomics (Önder et al., 2013) and natural isotope determination (Heylen et al., 2019), little recent progress has been made in developing this promising approach into a robust field tool for tick-host identification.

***Ixodes ricinus* and host immunity**

Another unresolved question in the field of tick research concerns the development of protective immunity against *I. ricinus* and whether this can be achieved by vaccination. The initial host response to tick bite has all the hallmarks of a typical defence mechanism against barrier breaches including pain and itching (stimulated by histamine and bradykinin), influx of inflammatory cells, complement activation, and initiation of wound repair, vasoconstriction and clotting to achieve haemostasis (Wikel, 2013). The result is an accumulation of eosinophils, macrophages, neutrophils, dendritic cells and some lymphocytes indicating a pronounced innate immune response during the first 24 hours of engorgement (Glatz et al., 2017). However, this response is rapidly counteracted by an arsenal of bioactive molecules in the tick's saliva that includes proteins, peptides and non-peptidic compounds. Transcriptomic studies have revealed a vast array of saliva proteins and peptides (the 'sialome') (Nuttall, 2019). Some are pre-formed in the unfed tick and stored in the salivary glands, ready to be released as soon as the tick starts to feed accounting for the immediacy of the reaction. While for many the function is as yet unknown, they are generally thought to have high affinity, selectivity and redundancy. Interestingly the sialome differs not only between tick species and life stages (Vancová et al., 2010) but even between individual ticks (Nuttall, 2019) and changes during the course of the engorgement (Glatz et al., 2017).

This extensive sialome diversity is thought to enable *I. ricinus* to evade the variety of immune defence mechanisms presented by the large range of host species it parasitises (Nuttall, 2019).

Salivary gland chemicals described for *I. ricinus* have histamine-binding and vasodilatory effects and inhibit alternative complement, neutrophil and classical macrophage activation (Brossard and Wikel, 2004; Wikel, 2013). Moreover, they suppress natural killer cell cytotoxicity and lymphocyte proliferation (Skallová et al., 2008). Several papers have also reported induced lymphocyte hypo-responsiveness (Hannier et al., 2003; Brossard and Wikel, 2004). The overall effect is pronounced T helper cell 2 (Th2) polarisation at the site of the bite (associated with raised IgE titres, mast cells and basophils, described in many laboratory animals) or the development of tolerance and a gradual down-regulation of the local response (described in humans) (Glatz et al., 2017). Importantly, this Th2 polarisation or tolerance at the bite site probably represents a key element in the transmission and infectivity of tick-borne pathogens.

However, some mammalian species develop resistance to *I. ricinus* in spite of the immunomodulatory effects exerted by tick saliva. Resistance to ticks has been described from certain ‘unnatural’ hosts such as rabbits, guinea pigs (Randolph, 1979) and sheep (Abdul-Amir and Gray, 1987), but also occurs in bank voles exposed to repeated tick infestation (Dizij and Kurtenbach, 1995). Ticks fed on resistant hosts exhibit pathological changes in their midgut, which are probably primarily mediated by antibody and complement (Nuttall, 2019). The microvilli become eroded, and endocytic mechanisms, protease and haemolysin activity are inhibited, leading to impaired digestion (Brossard and Papatheodorou, 1990). Affected immature ticks may detach prematurely and die, while adult females may be less able to convert their blood meal into eggs and produce smaller egg batches (Brossard and Papatheodorou, 1990). Ticks that remain attached, may feed very slowly or not at all and die in situ gradually becoming enveloped by the host's skin (D'Amico et al., 2017; Terhivuo et al., 2007).

In spite of considerable research efforts, the mechanisms that drive rejection responses in resistant hosts are still not fully understood as local immune-cell infiltrations largely resemble immune-cell profiles in susceptible hosts (Rego et al., 2019; Girardin and Brossard, 1989). Previous suggestions that production of IgG (in addition to IgE) may be the hallmark of the immune response of a tick-resistant host (Christe et al., 1998), have been discounted as they have also been detected in non-resistant human hosts. In fact anti-tick saliva IgG can be used to determine a patient's prior exposure to ticks (Nebreda et al., 2004). What does seem to differentiate resistant from susceptible hosts is that the former produce a pronounced delayed type hypersensitivity response and also significantly more histamine (as a result of cutaneous basophil hypersensitivity) at the site of the tick bite (Christe et al., 1998; Rego et al., 2019). Both of these responses are likely to enhance itching and grooming behaviour. Moreover, the production of histamine itself is likely to have a direct effect particularly on newly attached ticks, which are particularly sensitive to it (Rego et al., 2019).

Many vaccine trials have sought to recreate the rejection responses seen in *I. ricinus*-resistant animals, not least because it is thought that development of resistance to ticks would also confer some level of protection against tick-borne pathogens (reviewed by de la Fuente et al., 2016 and Rego et al., 2019). However, currently there is only a single commercial tick vaccine (marketed as TickGARD or Gavac). The vaccine contains recombinant *Rhipicephalus microplus* midgut antigens and reduces the number, weight and reproductive capacity of engorged *R. microplus* females (Contreras et al., 2019) but has no effect on other tick species including *I. ricinus* (Knorr et al., 2018). One of the most successful vaccine trials against *I. ricinus* using native soluble proteins extracted from midgut and salivary glands of partially fed females showed for the first time that immunisation of cattle against *I. ricinus* is possible in principle (Knorr et al., 2018). However, the tissue response to subsequent tick bites was so pronounced that it might be more harmful than beneficial, particularly in cases of heavy tick infestation. Regarding recombinant vaccine antigens, only a handful (including serine protease inhibitor [serpin]; cathepsins D, B, L, C plus legumin, ferritin 2 protein and aquaporin) have been shown to affect feeding or mortality of *I. ricinus*, and in most cases the effect was small and inconsistent (de la Fuente et al., 2016; Contreras and de la Fuente, 2017; Knorr et al., 2018; Rego et al., 2019). While they and many of the other vaccine candidates also caused a reduction in adult female engorgement weight and fertility, the effect was too subtle to warrant commercialisation.

Water management of *Ixodes ricinus* in the off-host phases

For ixodid ticks, which usually feed only 3 times in their life, maintaining both energy and water balance is a considerable challenge. Our knowledge of the water management of off-host ticks is based on a rather small number of studies, mostly dating back several decades reviewed by Knülle and Rudolph (1982) and Needham and Teel (1986, 1991). Most investigations were dedicated to measuring net water loss rates and survival of various tick species at different constant relative humidities. Various adaptations used to restrict water loss were also described, including producing guanine as the final excretory product, an epicuticle that distinctly decreases the rate of integumental water loss, spiracles that can be intermittently closed, the ability to absorb water by active water vapour uptake and ‘drinking’, and the ability to locate nearby places with a higher relative humidity when partially dehydrated. The maintenance of water balance is a fundamental determinant of survival for ixodid ticks and thus for their geographical distribution, which in a climate change scenario, is an increasingly important aspect of *I. ricinus* biology (Poretta et al., 2013). However, despite its importance, not much attention has been paid to this topic in modern research.

During feeding, ticks ingest, over a few days, relatively large volumes of host blood, which consists of approximately 80% water. Although water is a precious resource during the off-host phases, the longevity of a tick after the blood meal is mainly limited by the amount of nutrients obtained during the meal. Consequently, there is an obvious conflict between taking a maximum amount of water from the host to avoid desiccation soon after the blood meal versus taking a maximum amount of nutrients to provide enough energy for the subsequent off-host phase, which is often extended. While it is known that feeding ixodid ticks finely regulate their relative water content by re-injecting much of the surplus water and ions (Na^+, Cl^-) in the host blood into the host (Kaufman and Sauer, 1982), nothing is known about the mechanisms that facilitate this delicate osmoregulation. However, the fact is that although the relative water content of freshly engorged ixodid ticks varies between species and life stages, it is surprisingly constant in fully engorged individuals of a given species and life stage (Kahl, 1989). However, even in an optimal scenario of a blood meal with a high water content and a low rate of net water loss in the subsequent off-host period, the amount of water absorbed during engorgement is not sufficient to guarantee sufficient body-water until the next blood meal. As a consequence, off-host ticks need to both restrict their losses of body water and to actively take up water between blood meals.

It may appear obvious that ticks drink water as do most other terrestrial animals, however, it is probably a rare event for many tick species to come into contact with liquid water. Moreover, it may be difficult for a small tick to withdraw its mouthparts once it has inserted them into a droplet of water because of the surface tension of the water. There are just a few isolated reports of ticks drinking from droplets of water (e.g., Wilkinson, 1953; Londt and Whitehead, 1972; Kim et al., 2017), while several researchers observed unfed, moderately dehydrated *I. ricinus* ticks refusing to drink or indeed avoiding contact with liquid water (Lees, 1946; Kahl and Alidousti, 1997; Kröber and Guerin, 1999). Active water vapour uptake from subsaturated air seems to be the by far most important way for ticks to compensate for water loss, especially when unfed (Knülle and Rudolph, 1982). During this process, the salivary glands, specifically (and probably exclusively) the agranular alveoli type I, produce a secretion of unknown composition that is extruded onto the external mouthparts and that absorbs atmospheric water vapour when the ambient relative humidity is above 80-90% (Rudolph and Knülle, 1974; McMullen et al., 1976; Needham and Coons, 1984; Kahl and Knülle, 1988; Kahl et al., 1990; Gaede and Knülle, 1997). Subsequently the water-enriched oral secretion is swallowed thereby achieving a net water gain. This mechanism is very effective, providing substantial amounts of net water gain within hours or days, and has a comparatively low energy demand (Fielden and Lighton, 1996).

Although the ability to absorb water vapour is lost after the blood meal or during moulting when the old salivary glands disintegrate and new ones are formed (Kahl et al., 1990), it seems that all unfed ticks, including the argasids, are able to replenish their water requirements in this way. Regarding engorged ticks, it is currently thought that *Ixodes*, *Haemaphysalis* and *Argas* spp. that are capable of entering a developmental diapause retain this ability (Kahl and Knülle, 1988). Interestingly, the agranular alveoli type I are the only salivary gland alveoli that maintain their gross morphology and seem to be functional during the whole engorged phase in *I. ricinus* larvae and nymphs. Nothing is known from other tick species or genera in this regard.

While it has been established that Gene's organ of argasid and ixodid ticks produces a lipid secretion that covers the eggs to help to restrict their water loss (Lees and Beament, 1947), the actual water management (including gains of water) of the egg stage of *I. ricinus* is unknown. The same is true for the two moulting phases of *I. ricinus* (larva to nymph, nymph to adult), the preovipositing and ovipositing female, and all unfed and engorged life stages during the winter (below 9°C). Moreover, we do not know anything about the water management of adult *I. ricinus* males on the host or their longevity. However, in order to model the life cycle of *I. ricinus* under varying field conditions and calculate R_0, the basic reproductive number, a good understanding of the mortality of the different life stages of *I. ricinus* under field conditions is essential. This information would also give us a deeper insight in the complex life cycle strategy of this tick in different developmental phases. Finally, it has been suggested that ticks infected with pathogens may differ in some of their physiological and behavioural attributes from uninfected ticks (Hermann and Gern, 2015), another intriguing aspect that requires further study.

Factors affecting *I. ricinus* abundance

The factors that determine the abundance of *I. ricinus* have been the subject of considerable debate for many years. Identifying these factors is of major importance in predicting abundance and for the consequent implementation of tick-borne disease prevention and control measures.

Most investigators attempt to assess tick abundance by dragging flags of suitable material across vegetation in order to capture questing *I. ricinus*, particularly nymphs, which are more uniformly distributed than larvae or adults. The resulting data can be an indirect measure of tick-borne disease risk (acarological index) and the method is inexpensive and convenient. However, the performance of the method is variable depending on the weather at the time of sampling, vegetation structure, vegetation humidity levels and differences in operator efficiency and procedure (sampling by time or area). Furthermore, and since only a small proportion of ticks in the questing phase will be active at any given time (Nyrhilä et al., 2020), the results obtained should only be regarded as a proxy for tick abundance. It is difficult therefore to produce genuinely quantitative data with this method and in spite of considerable efforts to rectify this situation, no widely accepted standardized approach exists to date (Newman et al., 2019; Kjellander et al., 2021). Despite its drawbacks, dragging or flagging remains the most commonly used method for the determination of questing *I. ricinus* abundance, particularly of the nymphal stage and especially in the context of infection risk.

Weather and climate

The considerable challenges for ixodid ticks in maintaining their water balance have already been discussed. It comes as no surprise therefore that humidity is the main factor determining the distribution of *I. ricinus* and explains to a large extent the relative absence of the species from many areas around the Mediterranean. Studies in Switzerland have shown that when dry weather causes the saturation deficit to rise, nymphal questing activity decreases (Perret et al., 2000; Perret et al., 2003), but elsewhere it has been reported that questing of all stages can persist in hot weather as long as the litter layer remains sufficiently moist (Gray, 1984). Rising global temperatures are thought to contribute to the growth of *I. ricinus* populations by extending the tick questing season into winter and improving the winter survival of hosts, especially deer (Gray et al., 2009; Li et al., 2016). However, climate change will also alter precipitation patterns, which in some areas will lead to droughts, resulting in increased tick mortality and possibly limiting the distribution of *I. ricinus*.

It is often assumed that cold weather tends to increase the mortality of overwintering *I. ricinus* populations, but this is simplistic and under normal conditions in northern Europe, the majority of the tick population overwinters successfully as unfed stages, with a smaller proportion overwintering as eggs, engorged larvae and engorged nymphs in developmental diapause. Field observations in Germany suggested that it was only when snow cover was absent at very low air temperatures (-15°C or even colder) that tick populations were adversely affected (Dautel et al., 2016). In Ireland it has been observed that under quasi-natural conditions, unfed larvae and nymphs showed high rates of survival at air temperatures of -10°C without snow cover for a short period, whereas most engorged larvae and nymphs in developmental diapause perished (Gray, 1981). The cold-hardiness of *I. ricinus* larvae has been investigated under laboratory conditions (Dautel and Knülle, 1997), and it was found that although winter-acclimatized engorged larvae could survive -10°C for several days, they were not resistant to freezing and high mortality was experienced when in contact with ice. At present it is not clear how such data can be extrapolated to the field.

In addition to influencing the survival of individual ticks, temperatures also have a role in determining the northern distribution of established populations of *I. ricinus*, mainly by affecting the time taken to complete development within a season. Jaenson and Lindgren (2011) used the vegetation growing season (VGS), defined as the average number of days per annum when the mean temperature exceeds 5°C, as a proxy for temperature effects on tick development in the field, and concluded that the distribution of *I. ricinus* in Sweden, based on questionnaire data, is limited to regions where the VGS exceeds 170 days per year. Similar figures were obtained by Hvidsten et al. (2020), who recently determined the location of the northernmost established *I. ricinus* population in Norway by direct observation of questing stages, and estimated the VGS at the maritime site involved to be 184 days. The lowest VGS of any site with an established tick population was 173 days. It is clear that more direct observations on tick populations in relation to local temperatures are required to refine these data. Increasing temperatures are probably also the main drivers for the spread of *I. ricinus* to higher altitudes in several European mountain ranges (Materna et al., 2005; Martello et al., 2014; Garcia-Vozmediano et al., 2020). The effects on development times and extension of the questing season are undoubtedly the main mechanisms involved, but the accompanying spread of both large and small hosts to higher altitudes is also important.

Hosts

The catholic feeding habits of *I. ricinus* suggest that a large range of hosts might impact tick abundance, but large animals, particularly ungulates, are considered the most important maintenance hosts for *I. ricinus* populations because adult ticks are mainly dependent on them for successful feeding. In most regions woodlands inhabited by deer therefore constitute the main *I. ricinus* habitat. However, in some areas cattle and sheep can serve as the primary hosts for all tick stages and maintain populations in the absence of deer and where no large ungulates are present, medium-sized hosts such as hares, hedgehogs and foxes may have a role in maintaining tick populations (Jaenson and Tälleklint, 1996; Gray, 1998; Kahl and Geue, 1998).

In most animal populations density-dependent mortality is an important means of population regulation and Randolph (1997) working on the African tick, *Rhipicephalus appendiculatus*, suggests that in ticks this might occur through the stimulation of immunity in the primary maintenance hosts and also by increasing the mortality rates of these hosts, mainly as a result of pathogen transmission. However, for *I. ricinus* and deer, there is as yet no evidence for either mechanism and at present the most important population regulation mechanism for *I. ricinus* appears to be the availability of adult tick feeding opportunities. In typical *I. ricinus* habitat this is likely to depend largely on the density of deer as has been demonstrated by several studies involving both exclusion of deer by fencing (Gray et al., 1992; Gray et al., 1999; Gilbert et al., 2012; Hofmeester et al., 2017a), and studies in unfenced woodland (Pichon et al., 1999; Jensen et al., 2000; Tagliapietra et al., 2011; Ruiz-Fons et al., 2012; James et al., 2013). However, the precise relationship between deer density and tick abundance is unclear. Hofmeester et al. (2017a) suggested that in habitats of high biodiversity tick abundance may be determined by the mere presence of deer rather than density. An additional level of complexity is added by predator activity, which tends to reduce tick abundance (Hofmeester et al., 2017b), but at present there is no clear explanation for this observation.

Thus variations in the host community may complicate the relationship between deer and tick abundance. It is also possible that the efficiency of the vegetation litter layer in retaining moisture for the survival of off-host ticks may override any host effects (Goldstein et al., 2018), so that in some habitats even a few deer may maintain a substantial tick population. Conversely, high densities of deer and/or other large herbivores may cause the habitat to become degraded resulting in reduction of vegetation litter, which might explain observations of decreased tick abundance in forest habitats grazed by sheep or cattle (Gassner et al., 2008; van Wieren and Hofmeester, 2016).

Even without consideration of such environmental influences and the roles played by other hosts, the predicted relationship between tick and deer abundance appears to be rather complex, as illustrated by a mathematical model based on data from Wales and Dorset in the UK (Dobson and Randolph, 2011). The most striking feature of their theoretical results is that 10 years may elapse before the expected effects of changing deer densities are seen, which suggests that short-term studies may come to misleading conclusions on this topic.

More and longer-term studies on the effects of deer density on habitat structure and tick abundance are required, and the development of more efficient methods for estimating deer densities is necessary. Information is also required on possible immunity of deer to ticks, the relative susceptibility to *I. ricinus* of different deer species (red, roe and fallow), and the proportion of immature tick stages that feed on deer in various habitats.

Biodiversity and infection dilution

The role of host diversity in the prevalence of infectious diseases has excited considerable debate (Keesing et al., 2010), and amongst tick-borne pathogens the spirochaetes, *Borrelia burgdorferi* sensu lato (s.l.), the cause of Lyme borreliosis, have attracted most attention in this regard. LoGiudice et al. (2003) in the US proposed that reduced host diversity tends to result in increased prevalence of infected *Ixodes scapularis* nymphal ticks, hence giving rise to an increased risk of Lyme disease. However, another study of the same tick-host-pathogen system (Ogden and Tsao, 2009) concluded that the risk of acquiring tick-borne diseases is not necessarily reduced by increased vertebrate biodiversity, mainly because disease risk is determined by infected tick density rather than the proportion of infected ticks, and this is dependent on tick host density, regardless of host reservoir competence for the pathogen. Two other studies (Randolph and Dobson, 2012; Wood and Lafferty, 2013) broadly agree with this conclusion, arguing that even if tick hosts that are not reservoir competent are added to an ecosystem, the risk of disease remains relatively unaffected.

I. ricinus is the main vector of *B. burgdorferi* s.l. in Europe, where the situation is further complicated by the varied habitats in which this tick species is found (Gray, 1998; Ruyts et al., 2016; Ehrmann et al., 2017) and also by the occurrence of several *Borrelia* genospecies utilizing different reservoir hosts and showing different levels of human pathogenicity (Stanek et al., 2012; Randolph and Dobson, 2012). Deer are important components of the host community in Lyme borreliosis habitats because they are the main maintenance hosts of *I. ricinus* populations, although they are not *B. burgdorferi* s.l. reservoirs. However, attempts to determine the effects of differing deer densities on infection rates in *I. ricinus* have produced inconsistent results. For example, in Ireland Gray et al. (1992) reported that low *B. burgdorferi* s.l. nymphal and adult tick infection rates occurred inside a 600-hectare site harbouring a high density of fallow deer (*Dama dama*), whereas outside the deer fence, but still within the wood, infection rates of the relatively scarce questing ticks were approximately seven times higher. The density of infected ticks outside the fence was estimated to be nearly twice that of the area on the deer side of the fence. Rosef et al. (2009) in Norway made similar observations on infection rates in adult and nymphal *I. ricinus*, which were found to be lower on islands with high densities of roe and red deer compared with mainland sites with lower deer densities. In contrast, Tälleklint and Jaenson (1996) found a positive relationship between tick abundance and infection rates in Sweden, and Pichon et al. (1999) reported no significant differences in tick infection rates in areas of French forest with differing densities of deer. None of these forest studies were able to consider the contributions of other tick hosts or of *B. burgdorferi* s.l. reservoirs, so the results are difficult to compare. However, a Belgian study that assessed the contribution of host communities to infected-tick abundance in 19 forest stands using point counts for birds, and camera and bait traps for mammals, concluded that there was little evidence for a relationship between the abundance of infected ticks and host diversity (Ruyts et al., 2018). A similar study in the Netherlands also failed to detect a dilution effect for a variety of tick-borne pathogens transmitted by *I. ricinus* to mammals (including *B. afzelii*, *B. miyamotoi*, *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis*), although there was apparently a dilution effect in relation to the encounter probability of ticks with wood mice (*Apodemus* spp.) for the bird-associated *B. garinii* and *B. valaisiana* (Takumi et al., 2019).

Several studies on cattle, which, like deer, are not reservoirs for *B. burgdorferi* s.l., demonstrate the importance of tick-host community composition in determining the abundance of *B. burgdorferi* s.l.-infected ticks. Whereas Sprong et al. (2020) found that cattle grazing within highly biodiverse forests in the Netherlands had little or no effect on the abundance of infected nymphal ticks, Gray et al. (1995) reported that in low-biodiversity grazings in the west of Ireland, infected ticks were very rare in areas where cattle or sheep were the main hosts for all tick stages. Similar results were obtained by Richter and

Matuschka (2006) when they compared infection rates in ticks collected from a fenced cattle pasture in France with those of ticks collected 10 m outside the pasture. This type of low-biodiversity tick habitat is not uncommon, especially in Britain and Ireland, where grass-fed livestock often predominate in tick habitats, in which cattle and sheep alone can maintain populations of *I. ricinus*. In such circumstances low biodiversity can evidently decrease rather than increase the abundance of infected ticks, contrasting with the dilution effect observed in parts of the US (LoGiudice et al., 2003), and emphasizing the variability of the effects of biodiversity on *B. burgdorferi* s.l.-infection risk of humans in Europe. The involvement in this complex topic of *I. ricinus* and the pathogens it transmits requires further study.

The transport of *Ixodes ricinus* and *Ixodes*-borne pathogens by migratory birds

In response to global warming, *I. ricinus* has extended its distribution at its northern edge (Jaenson et al., 2012; Hvidsten et al., 2020) and in altitude (Materna et al., 2005; Martello et al., 2014). While much of this advance is probably due to a gradual local step-by-step process, much larger distances may be covered by birds carrying ticks and tick-borne pathogens over hundreds or even thousands of kilometres within short time periods in spring and autumn. The main eco-epidemiological question is whether or not this is likely to create new, distant foci of tick-borne disease.

Birds, including migratory birds, commonly feed immature *I. ricinus* ticks and may transport them, and the pathogens they carry, to areas where the pathogen has not occurred so far, even if the birds themselves do not become infected. According to a growing number of scientific articles this may create a new tick-borne disease risk in these areas (Geller et al., 2013; Hasle, 2013; de la Fuente et al., 2015; Mărcutan et al., 2016; Pedersen et al., 2020). However, to our knowledge there are still no published records of the successful establishment of *Ixodes* ticks resulting from long-distance transport by migratory birds. Existing studies are usually restricted to counting ticks on birds and sometimes also checking the infection status of the ticks. But this does not take into account the questions of whether the habitat that the tick has been transported to will support its survival, whether the local photoperiodic conditions are suitable for its development to the next life stage, or whether it is likely to find a suitable host that is susceptible to the pathogen(s) it carries. It is, however, noteworthy that the recent occurrence of TBE virus on Haapasaari island, which is part of the Finnish Kotka archipelago in the Baltic Sea, could be caused by long-distance bird transportation over several hundred kilometres. The Siberian subtype of the TBE virus was found in *I. ricinus* ticks there, although the common vector of that subtype, *I. persulcatus*, seems to be absent (Jääskeläinen et al., 2015).

The specific habitat requirements of *I. ricinus*, particularly with regard to microclimatic humidity conditions, are well known. However, because between April and August, daylength in the southern distribution areas of *I. ricinus* is distinctly shorter than in the north, regional adaptations with regard to photoperiodism are required. The effects of local photoperiodic conditions (external cues that regulate the seasonal development from larva to nymph and from nymph to adult and also affect seasonal questing behaviour) on newly arrived ticks from other regions are not understood at all. In central Europe, *I. ricinus* larvae and nymphs engorged in July and August, respectively, switch from a direct development to the next life stage to a developmental diapause (Chmela, 1969; Bauch, 1971; Kahl, 1989). This is the result of long-term selection due to the fact that from a certain timepoint in the year onwards, temperatures are normally not high enough to allow the engorged larval or nymphal tick to complete its development to the following life stage before winter, and moulting ticks might not be able to survive the winter (O. Kahl, unpublished). In north European *I. ricinus* populations, this seasonal switch might occur distinctly earlier in the season than in the south because of the lower temperatures and the shorter growing season. Diapausing engorged larvae and nymphs overwinter and moult to the following life stage only in the following summer. Therefore, a southern tick that has been transported north, or a northern tick that has been transported south would receive seasonal misinformation and may be stimulated to undergo developmental diapause at the wrong time of year – if at all –, jeopardizing their survival. In addition to quantitative differences (varying photoperiodic thresholds or critical photoperiods) there may also be qualitative differences in the innate photoperiodism mechanism between southern and northern populations. Moreover, unfed *I. ricinus* are also photosensitive (Belozerov, 1982; Gray et al., 2016), and the distinctly longer days in late spring and summer in the north may provide seasonal misinformation to the unfed stages of ticks originating in the south and vice versa, e.g. their propensity to quest.

Ticks from different geographical regions may be adapted to different temperatures, for example, Gilbert et al. (2014) demonstrated that ticks of northern origin in western Europe can quest at lower temperatures than those from the south.. It is likely that the temperature-dependence of the development to the next life stage also varies among different geographical populations. Temperature and photoperiod are known to have combined effects in the induction and manifestation of behavioural and developmental diapauses (Belozerov, 1982), but we know almost nothing about the effects of these influences in the field.

Another feature of importance for the survival of feeding ticks transported to a new latitude by birds, is that once replete they need to drop-off into suitable habitat for development to the next stage, because they have very restricted mobility. Kheisin and Lavrenenko (1955) demonstrated that drop-off is not a random event in a study of female *I. ricinus* on cattle maintained in a 12:12 hour field/stall regime. Once engorged, the ticks always dropped off in the field, never in the stall, irrespective of the time of day. Clearly, the stimuli to instigate drop-off are crucial for the survival of bird-transported ticks, as they are in many other scenarios, but at present their identity is unknown.

For *I. ricinus*, which already occurs right across Europe, it is very difficult to determine whether new arrivals from other, distant parts, survive and even more difficult to assess the eco-epidemiological risk associated with the tick-borne agents carried by them. There are several scenarios dependent on whether or not the migratory bird that is carrying ticks is reservoir-competent for the particular tick-borne pathogen:

(1) If the bird is reservoir-competent for the pathogen, with long-lasting infectivity, it may transmit its infection to feeding ticks both during flight and after arrival. In this case a new natural focus of disease may develop, but on a larger scale this is dependent on the number of infected birds involved.

(2) If the bird is reservoir-competent for the pathogen but with short-lasting infectivity, it may infect only a few ticks. In this case the chance that a new natural focus of disease develops appears low.

(3) If the bird is not reservoir-competent for the given pathogen, it will not have a direct role in the maintenance of the pathogen. Any larvae that these birds transport will only be infectious if they have been infected transovarially, in which case an individual larva can later infect up to two further hosts and thereby create a new focus of disease. However, this is contingent on their survival and development to the nymphal stage.

(4) An infected nymph that has been transported by a migratory bird and manages to develop to the adult stage after being deposited can infect one more host.

There are clearly significant gaps in our knowledge regarding the role of migratory birds, and further studies that compare southern, central and northern *I. ricinus* populations in their basic biological characters and investigate their ability to survive in distant areas along the known routes of migratory birds are needed. Where possible, these studies should use naturally increasing and decreasing daylengths (and not only artificially static long-day or short-day conditions) and should focus in particular on the varied combined influence of temperature and photoperiod on the seasonality of development (moulting, oviposition) and questing in different life stages of *I. ricinus* in their home regions and in distant areas. Regarding the potential threat of new foci of *Ixodes*-borne disease becoming established by tick transport on migratory birds, future research could focus on the discontinuous distribution of *Ixodes*-borne pathogens and genotypes associated with bird fly-ways or investigate the possible predominance of bird-associated tick-borne microbes (e.g. *Borrelia valaisiana*) in *I. ricinus* on the leading edge of their expanding range, e.g. in northern Europe. Another interesting approach would be to survey islands that lack tick maintenance hosts such as deer or livestock and that might serve as staging posts for migratory birds for the presence of questing ticks and tick-borne pathogens.

Genetic variation in *I. ricinus*

The question of whether ecologically-distinct phenotypes differ genetically is still unresolved. *Ixodes ricinus* was the subject of the first population genetics studies of any tick species, which concerned two isozyme loci in ticks from four locations in southern Ireland. Both loci showed high diversity. One (for alpha-glycerophosphate) was associated with walking and resistance to desiccation but not to location (Healy, 1979a), whereas the other (for phosphoglucomutase) showed some spatial polymorphism, but was not associated with specific biological features (Healy, 1979b). Unfortunately, the findings of these promising studies were not confirmed in a more recent investigation in Switzerland that focused on the same allozymes (Delaye et al., 1997). Variation is more likely to occur on a much larger geographic scale and the first such study of *I. ricinus* mapped differences in cuticular hydrocarbons between ticks from different regions of Europe (Estrada-Peña et al., 1996). Ticks from three of the seven discriminated populations were then compared for susceptibility to *Borrelia afzelii* using gerbils as experimental hosts (Estrada-Peña et al., 1998). Differences in both tick infection rate and intensity were observed, but absolute standardization among the three laboratories involved in this small-scale experiment was difficult. Furthermore, it was not clear whether the observed cuticular hydrocarbon variation reflected genetic or environmental effects. As discussed above, one of the few studies to date that compared the biology of *I. ricinus* from different regions found that ticks from cool climates become active and quest at lower temperatures than those from relatively warm ones (Gilbert et al., 2014). Interestingly, the greatest differences were apparent between ticks from regions where they also appear to exhibit different cuticular hydrocarbon patterns (Estrada-Peña et al., 1996), suggesting that these variations may have a genetic basis.

Attempts to demonstrate genetic differences between separate populations of *I. ricinus* have generally been disappointing (Delaye et al., 1997; Casati et al., 2008; Noureddine et al., 2011), although Dinnis et al. (2014) clearly differentiated *I. ricinus* specimens from Britain and Latvia by multilocus sequence typing of mitochondrial genes, and Røed et al. (2016) demonstrated three separate lineages in Britain and Scandinavia, also using mitochondrial genes. The resulting map of the lineages in the latter study resembles that for the cuticular hydrocarbon groups in certain respects, again suggesting that the latter might have a genetic basis. This implies that in addition to differences in low temperature limits for questing, there are genetically-determined regional differences in *I. ricinus* susceptibility to certain pathogens.

There has also been some speculation that host specificity might vary geographically. Gray et al. (1999) suggested that the low frequency of nymphs on rodents in Ireland compared with continental Europe might have a genetic basis, perhaps explaining the rarity of the rodent-associated *B. afzelii* in Ireland, despite obvious contact between rodents and nymphal *I. ricinus*. The heavier burdens of subadult *I. ricinus* on deer in Scotland compared with those in the Netherlands (Hofmeester et al., 2017) may also have a genetic basis, although the authors speculated that the difference was due to more abundant and diverse hosts in the Netherlands. The apparent emergence of genetically-distinct *I. ricinus* races with specific host preferences, where older established tick populations are more likely to show host specialisations (McCoy et al., 2013), may be relevant in both these cases.

As suggested in the previous section, the expression of diapause may vary in ticks from different latitudes since photoperiodism is a major cue for this phenomenon. Although diapause phenomena are now well known in *I. ricinus* (Belozerov, 1982; Gray et al., 2016), the subject has received uneven attention across Europe and little is known about geographical variations. However, Belozerov (1982) reported that the critical photoperiod (the day length that elicits diapause in 50% of the population) of engorged larvae was substantially shorter in Moldavia (46.3°N) than in a northern population in St. Petersburg (59.6°N), and in the closely related *I. persulcatus*, regional differences in diapause-induction dates for larvae and nymphs have been observed (Korotkov, 2009). It is highly likely that other geographical differences occur but at present diapause phenomena in ticks attract little research attention.

These observations on apparent phenotypic variation are encouraging, and it remains to be seen whether the increasing interest in genetic studies of *I. ricinus* will result in more evidence for genetically structured populations showing differences in biology that have significant implications for disease eco-epidemiology.

As a result of the poorly understood intraspecific genetic variation in ticks there is also currently no standard PCR assay for tick species identification or subtyping. The most commonly used locus is the 16S ribosomal (16S rRNA) gene as it is thought to be informative at the genus and species, though not at the family and subfamily levels (Xu et al., 2003). It also has a good sequence recovery rate (Lv et al., 2014b), and GenBank provides sequence information for most tick species. Other frequently targeted mitochondrial genes include the cytochrome oxidase subunit 1 (COI) and the 12S rRNA genes. COI is generally recognised as the standard marker for DNA barcoding of all animal species and can be used to reliably discriminate between tick species (Lv et al., 2014a), however, amplification and sequencing of this locus can be difficult (Lv et al., 2014b). Nuclear gene regions such as the 18S rRNA and the internal transcribed spacers 1 or 2 (ITS1 and ITS2) are also sometimes used, the latter particularly for species subtyping as it has high intraspecific diversity. However, for many tick species, sequence information for these loci is not available on GenBank.

Further studies on intraspecific genetic variation will also facilitate the discrimination of races, subspecies or even new, actual species within the *I. ricinus* complex. The identification of *I. inopinatus*, until recently considered to be synonymous with *I. ricinus* (Estrada-Peña et al., 2014) is an example of how a separate species or subspecies, possibly with differing biological characteristics, can remain hidden for many decades.

***Ixodes ricinus* and its microbiome**

Facilitated by recent advances in DNA sequencing technologies, much research has focused on the tick microbiome and its potential effects on the acquisition, survival and transmission of tick-borne pathogens. One of the major groups of microbiome organisms are maternally-derived intracellular endosymbionts. In *I. ricinus* these include *Candidatus* Midichloria mitochondrii, an alphaproteobacterial symbiont that may serve as an ATP source during oogenesis (Bonnet et al., 2017) and is commonly found in the mitochondria of ovarian cells (Sassera et al., 2008), *Coxiella*- and *Rickettsia*-like endosymbionts and *Rickettsiella*, *Spiroplasma* and *Wolbachia* species (Duron et al., 2017). However, *Wolbachia* as well as the occasionally reported *Arsenophonus* spp. (Narasimhan and Fikrig, 2015) may actually be endosymbionts of *Ixodiphagus hookeri*, an endoparasitoid wasp that infects the tick rather than a symbiont of the tick itself (Plantard et al., 2012; Bohacsova et al., 2016).

In addition to endosymbionts a vast array of mutualists, commensals and potential pathogens colonise external and internal surfaces, particularly the midgut, where they may be associated with biofilm (Narasimhan et al., 2017). These microorganisms are thought to be acquired directly from host skin during feeding or from soil or plants via spiracles, or oral and anal pores (Narasimhan et al., 2017; Estrada-Peña et al., 2018). As a result, there are pronounced differences in the microbiome composition of tick populations from different locations and between life stages and individuals in the same population (Duron et al., 2017). However, the fact that different methods have been used in studies on the microbiome of *I. ricinus* make them difficult to compare. A metagenomic (V6-16S rRNA) analysis of adult and nymphal *I. ricinus* ticks revealed a total of 108 genera belonging to representatives of all major bacterial phyla (mostly Proteobacteria, followed by Actinobacteria or spirochaetes, with Firmicutes and Bacteriodetes being least prevalent) (Carpi et al., 2011), while pyrosequencing followed by BLSOMs analysis (Batch Learning Self-Organising Maps) of adult females identified 133 bacterial/archaeal genera belonging to 22 bacterial phyla with Gammaproteobacteria, Firmicutes, Alphaproteobacteria, Planctomycetes, spirochaetes, Bacteroidetes, Epsilonproteobacteria, Deferribacteres and Elusimicrobia listed as the most common ones (Nakao et al., 2013). Nymphs and adult males generally have a more diverse microbiome than larvae as they gradually accumulate microorganisms throughout their lives. In contrast, alpha diversity in adult females is typically lower, possibly because they have higher relative burdens of endosymbionts prior to transovarial transmission (van Treuren et al., 2015).

The various tick-borne pathogens that are transmitted by *I. ricinus* survive the long periods between feeds sequestered in various specific tissues. For example, *B. burgdorferi* s.l. spirochaetes remain in the midgut during the digestion of the blood meal and moulting. Once the tick starts to feed again, they multiply rapidly in the newly engorged blood, cross the midgut barrier and enter the haemolymph and salivary glands. Adhesion to midgut cells, subsequent dissemination and invasion of the salivary glands is facilitated by various receptors and ligands such as the *Borrelia* outer surface proteins A and C and the corresponding tick receptor TROSPA and the salivary gland protein Salp15. To date these ligands have only been identified in *Ixodes scapularis* (Pal et al., 2004; Ramamoorthi et al., 2005) although genes encoding similar proteins have been described in *I. ricinus* (Figlerowicz et al., 2013; Liu et al., 2014).

Interactions between *B. burgdorferi* s.l. and the rest of the microbiome are a sensitive balancing act mediated by the tick's immune system and have so far only been described for *I. scapularis*. On the one hand, spirochaetes in the tick midgut stimulate expression of a tick protein PIXR, which helps to control the formation of bacterial biofilm presumably preventing overgrowth (Narasimhan et al., 2017). On the other hand, the presence of a healthy microbiome is essential for successful colonisation of the midgut by *B. burgdorferi* s.l. (Narasimhan et al., 2014). The reason for this is that the presence of the tick midgut microbiome stimulates secretion of peritrophin, a major component of the peritrophic matrix (PM), a glycan-rich mucus-like layer thought to be analogous to the gut mucosa in vertebrates. Spirochaetes typically invade the space between the PM and the epithelium (Narasimhan et al., 2014). In contrast, *A. phagocytophilum* requires a thin and permeable PM for successful invasion and colonisation as it rapidly passes from the midgut to the salivary glands where it is detectable within 24 hours of engorgement (Liu et al., 2011). Again, evidence from other tick species indicates that this change is affected by a complex interplay between the vector, the pathogen and the rest of the microbiome (Abraham et al., 2017). In *I. scapularis*, the bacterium induces expression of an antifreeze glycoprotein IAFGP, which has antimicrobial properties, particularly against Gram-positive bacteria thereby disrupting biofilm formation (Heisig et al., 2014). The resulting alteration of the gut microbiome in turn affects the integrity of the PM, which becomes thinner and more permeable (Abraham et al., 2017). As the PM in *I. ricinus* is not fundamentally different from that of *I. scapularis* (Zhu et al., 1991) similar mechanisms may be at play in this species. However, whether they are also mediated by a glycoprotein that has antifreeze properties is doubtful as, contrary to *I. scapularis* (Neelakanta et al., 2010), no association between *A. phagocytophilum* infection status and cold resistance has been reported for *I. ricinus* (Lejal et al., 2019). Neither IAFGP nor salivary gland proteins P11 or Salp16, which in *I. scapularis* facilitate invasion of haemocytes and salivary glands, respectively (Liu et al., 2011), have been reported in *I. ricinus* to date.

Very little research has focused on the interactions between *Babesia* spp., *Ixodes* ticks and their microbiome. On entering the tick midgut, the parasites undergo sexual development resulting in the formation of zygotes that penetrate the PM and invade the midgut lining. Early reports that zygotes preferentially invade specific vitellogenic gut epithelial cells (Agbede et al., 1986) have not been confirmed, however, it has been shown that a vitellogenin receptor that facilitates uptake of the yolk protein by developing oocytes is used by *Babesia gibsoni* to gain access to the developing eggs in the ovaries of *Haemaphysalis longicornis* (Boldbaatar et al., 2008). Transovarial transmission also occurs in *Babesia divergens,* but whether it utilizes the same mechanism to infect the ovaries or whether, like some endosymbionts, it is transmitted to the offspring of *I. ricinus* via bacteriocytes (Neelakanta et al., 2010) is not known. Other tick receptors that are thought to facilitate colonization of *Rhipicephalus microplus* and *Rhipicephalus annulatus* with *Babesia* spp.

include homologues of TROSPA, subolesin, serum amyloid A and calreticulin (Antunes et al., 2012; Merino et al., 2011). With the exception of serum amyloid A, homologues of these proteins have been identified in *I. ricinus* (Figlerowicz et al., 2013; de la Fuente et al., 2006; Vu Hai et al., 2013), however, their potential role in the acquisition or transmission of *Babesia* has not yet been described.

The relationship of other tick-borne agents such as *Rickettsia* spp. with closely related commensal endosymbionts in the tick microbiome might be chiefly determined by competition between the two (Narasimhan and Fikrig, 2015). It is obvious that a lot of work is still required to understand the complex and highly specific interactions between *I. ricinus*, its microbiome and a given tick-borne pathogen. Yet, this knowledge could provide the key to reducing the suitability of ticks as pathogen vectors.

Conclusions

The long history of research on *I. ricinus* suggests that there may not be many gaps in our knowledge of the biology of this species. Our original concept for this review therefore was a relatively short document highlighting aspects of *I. ricinus* biology that had received insufficient attention, that had proved particularly difficult to elucidate or that consisted of new and undeveloped findings. However, it soon became apparent that there are still many topics to be considered. These range from basic biology such as water management, first investigated in detail more than 70 years ago, to complex ecological problems such as the role of deer in tick abundance and the impact of biodiversity on pathogen transmission, and to exciting new research resulting from cutting-edge technologies, such as genomics, studies on the microbiome and vaccine technology. Many of the important remaining questions will require long-term projects rather the typical short, one-off studies facilitated by the current funding landscape. We have tried to shape and define as yet unanswered questions and to raise new ones in the hope of providing emphasis and direction for future research on *I. ricinus.*

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