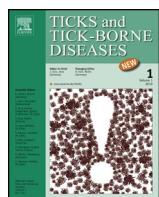




Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbd

Original article

Genetic variation in transmission success of the Lyme borreliosis pathogen *Borrelia afzelii*



Nicolas Tonetti^{a,1}, Maarten J. Voordouw^{b,*1}, Jonas Durand^b, Séverine Monnier^a, Lise Gern^a

^a Institute of Biology, Laboratory of Eco-Epidemiology of Parasites, University of Neuchâtel, Neuchâtel, Switzerland

^b Institute of Biology, Laboratory of Ecology and Evolution of Parasites, University of Neuchâtel, Neuchâtel, Switzerland

ARTICLE INFO

Article history:

Received 24 September 2014

Received in revised form

27 December 2014

Accepted 16 February 2015

Available online 4 March 2015

Keywords:

Borrelia afzelii

Co-feeding transmission

Ixodes ricinus

Lyme borreliosis

Reproductive number

Vector-borne pathogen

ABSTRACT

The vector-to-host and host-to-vector transmission steps are the two critical events that define the life cycle of any vector-borne pathogen. We expect negative genetic correlations between these two transmission phenotypes, if parasite genotypes specialized at invading the vector are less effective at infecting the vertebrate host and vice versa. We used the tick-borne bacterium *Borrelia afzelii*, a causative agent of Lyme borreliosis in Europe, to test whether genetic trade-offs exist between tick-to-host, systemic (host-to-tick), and a third mode of co-feeding (tick-to-tick) transmission. We worked with six strains of *B. afzelii* that were differentiated according to their *ospC* gene. We compared the three components of transmission among the *B. afzelii* strains using laboratory rodents as the vertebrate host and a laboratory colony of *Ixodes ricinus* as the tick vector. We used next generation matrix models to combine these transmission components into a single estimate of the reproductive number (R_0) for each *B. afzelii* strain. We also tested whether these strain-specific estimates of R_0 were correlated with the strain-specific frequencies in the field. We found significant genetic variation in the three transmission components among the *B. afzelii* strains. This is the first study to document genetic variation in co-feeding transmission for any tick-borne pathogen. We found no evidence of trade-offs as the three pairwise correlations of the transmission rates were all positive. The R_0 values from our laboratory study explained 45% of the variation in the frequencies of the *B. afzelii* *ospC* strains in the field. Our study suggests that laboratory estimates of pathogen fitness can predict the distribution of pathogen strains in nature.

© 2015 Elsevier GmbH. All rights reserved.

Introduction

The ability to establish an infection in a naive host and transmission to secondary hosts are the critical fitness components of the parasite life cycle. Parasite populations often exhibit genetic variation in life history traits despite the fact that these characters are expected to be under strong selection. Life history theory suggests that negative genetic correlations (trade-offs) between different components of the parasite life cycle can influence the evolution of the optimal parasite phenotype (Stearns, 1992). Previous work has shown trade-offs among a variety of parasite life history traits

including within- and among-host transmission, ability to avoid clearance by the host immune system, and parasite life expectancy (de Roode et al., 2008; Ebert, 1998; Fraser et al., 2007; Mackinnon et al., 2008; Mackinnon and Read, 1999). These trade-offs are of considerable interest because they drive the evolution of virulence, which is the level of parasite-induced harm to the host (Ebert and Bull, 2003).

Life history trade-offs might be particularly prevalent in vector-borne pathogens that are adapted to live in two very different environments: an arthropod vector and a vertebrate host. The life cycle of all vector-borne pathogens contains two critical transmission events: vector-to-host transmission and host-to-vector transmission (Randolph, 1998). Vector-to-host transmission requires the pathogen to colonize the transmission tissues of the vector (often the salivary glands) and avoid clearance by the vertebrate immune system. Host-to-vector transmission requires ingestion of the pathogen by the vector from the host tissues (blood, skin) and resistance against the arthropod immune system. The genetic and physiological mechanisms underlying these two

* Corresponding author at: Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland. Tel.: +41 32 718 3114; fax: +41 32 718 3001.

E-mail addresses: nicolas.tonetti@icloud.com (N. Tonetti), maarten.voordouw@unine.ch (M.J. Voordouw), jonas.durand@unine.ch (J. Durand), severine.monnier@unine.ch (S. Monnier), lise.gern@unine.ch (L. Gern).

¹ These two authors contributed equally to this work.

transmission events are likely to be very different (Tsao, 2009). For example, pathogen interactions with vector saliva influence the efficacy of vector-to-host transmission (Titus and Ribeiro, 1988; Wikle, 1999) whereas pathogen load in the vertebrate host determines host-to-vector transmission success (de Roode et al., 2005; Raberg, 2012). Given the difficulty of adapting to both vector and host, we expect vector-borne pathogens to exhibit trade-offs between vector-to-host and host-to-vector transmission success. In the present study, we test this hypothesis using the tick-borne pathogen, *Borrelia afzelii*.

The tick-borne spirochete bacterium, *B. afzelii*, is one of the most important causes of Lyme borreliosis in Europe (Piesman and Gern, 2004). This pathogen uses the tick, *Ixodes ricinus*, as its vector and a variety of rodent species as its reservoir hosts (Humair and Gern, 1998; Humair et al., 1995; Kurtenbach et al., 1998). *B. afzelii* establishes a chronic and systemic infection inside rodent hosts. Studies on *B. afzelii* have found that host-to-tick transmission rates were high over the lifetime of the infection (Gern et al., 1994; Humair et al., 1999). In contrast, studies on *B. burgdorferi* sensu stricto (s.s.) have found that host-to-tick transmission can decline rapidly over short time periods (i.e. one month) (Derdakova et al., 2004; Hanincova et al., 2008; Lindsay et al., 1997). In addition to the classic mode of systemic transmission, *B. afzelii* is also capable of co-feeding transmission (Gern and Rais, 1996; Hu et al., 2003; Richter et al., 2002). Co-feeding transmission occurs when the pathogen is transmitted directly between vectors that are feeding on the same host at the same time and can occur in the absence of systemic infection (Randolph et al., 1996; Voordouw, 2015). A recent field study suggested that strains of *B. afzelii* may differ in their efficacy of co-feeding transmission (Pérez et al., 2011). These findings motivated us to test whether the efficacy of co-feeding transmission differed among strains of *B. afzelii* in the present study.

Local populations of *Borrelia* pathogens often contain a number of genetically diverse strains (Pérez et al., 2011; Qiu et al., 2002). Previous genetic work has shown that there is very little horizontal gene transfer and that *Borrelia* strains are essentially clonal (Bunikis et al., 2004; Dykhuizen and Baranton, 2001; Hellgren et al., 2011; Qiu et al., 2004). The *ospC* gene, which codes for outer surface protein C (OspC), is a commonly used genetic marker to differentiate among strains (Wang et al., 1999). Strains differing at their *ospC* genotype have been compared with respect to a number of different phenotypes expressed in the vertebrate host including spirochete load, pathology, antibody profiles, and host-to-tick transmission (Baum et al., 2012; Derdakova et al., 2004; Hanincova et al., 2008; Wang et al., 2001, 2002). In the present study, the *ospC* gene is of particular interest because the OspC protein plays a critical role during the tick-to-host transmission event (Radolf and Caimano, 2008; Tilly et al., 2008). Mutant strains lacking the *ospC* gene are unable to colonize the tick salivary glands (Fingerle et al., 2007; Pal et al., 2004) and/or the vertebrate host (Grimm et al., 2004; Tilly et al., 2006). Thus in the present study, the *ospC* gene is both a potential virulence factor and a strain-specific genetic marker.

The purpose of this study was to test whether there was genetic variation and covariation among strains of *B. afzelii* in the three canonical fitness components of any tick-borne pathogen: tick-to-host transmission, host-to-tick (systemic) transmission, and tick-to-tick (co-feeding) transmission. As *B. afzelii* is a species that is specialized on rodents (Piesman and Gern, 2004), we used laboratory mice as an approximate model of the wild rodent reservoir host. Our laboratory colony of *I. ricinus* was used as the tick vector. For each *B. afzelii* strain, we combined the transmission components into a single estimate of the reproductive number (R_0) using recently developed next generation matrix models (Harrison and Bennett, 2012; Harrison et al., 2011; Hartemink et al., 2008). R_0 measures the ability of a pathogen to invade and persist in the host

population and provides a convenient framework for comparing fitness between pathogen strains. Theory predicts that in the absence of inter-strain competition, each strain will rise to a frequency that is commensurate with its R_0 value (Gupta et al., 1998). Recent work by our group estimated the frequencies of *B. afzelii* strains (using the *ospC* gene as a marker) in wild *I. ricinus* ticks at two sites in Switzerland (Pérez et al., 2011). In the present study, we tested whether our strain-specific estimates of R_0 in laboratory mice were predictive of the frequencies of the *B. afzelii* strains in the field.

Materials and methods

Mice and ticks

Pathogen-free, one month-old, male BALB/c mice were used in this study and were housed in separate cages. Prior to any invasive procedures (inoculation of spirochetes, tick infestations, and ear biopsies), mice were anaesthetized with a mix of xylazine (Xylasol®, 10 mg/kg) and ketamine (Narketan®, 100 mg/kg) to minimize discomfort. Tick infestations and ear biopsies were attempted only after the loss of toe pinch reflexes. Larval and nymphal ticks came from the laboratory colony of spirochete-free *I. ricinus* ticks of the University of Neuchâtel and were reared according to Graf (1978). Infected nymphal ticks were used one month after moulting and xenodiagnostic larval ticks were used at least one month after hatching. All experiments involving mice respected the Swiss legislation on animal experimentation and were authorized by the Veterinary Service of the Canton of Neuchâtel (Authorization numbers 1/2006 and 2/2009).

Borrelia afzelii isolates

Most of the *B. afzelii* isolates used in this study came from the isolate collection of the University of Neuchâtel (Table 1). These isolates were obtained from ticks and rodent ear biopsies that had been collected at two different sites in Switzerland: Glütschbachtal (Thoune, Bern, Switzerland) and Bois de l'Hôpital (Neuchâtel, Switzerland). All isolates had been passaged fewer than five times to avoid the loss of the virulence genes that are critical for infection. However, it is possible that our low-passage treatment reduced the infectiousness of the cultured isolates (Ebert, 1998). Strains E61 and P/sto were obtained from the Pasteur Institute, Paris because our Swiss collection of *B. afzelii* isolates did not contain *ospC* groups A3 and A4 (Table 1).

The *B. afzelii* isolates were selected to represent a diversity of *ospC* groups. The nomenclature of the *B. afzelii* *ospC* groups used in the present study was developed by Lagal et al. (2003) and was also used in the study by Pérez et al. (2011). We note that Bunikis et al. (2004) developed a different nomenclature for the *B. afzelii* *ospC* groups that is also used in the literature (Table 1). All *ospC* groups used in the present study are known to infect wild rodents (Pérez et al., 2011). The isolates with *ospC* group YU were of particular interest because they appear to have high co-feeding transmission (Pérez et al., 2011). We used experimental infection via needle inoculation (see below) to determine which isolates were infectious for BALB/c mice and retained eight isolates belonging to six *ospC* groups: NE4053 (A1.v1), NE5046 (A1.v2), NE36 (A2), E61 (A3), P/sto (A4), NE4054 (ME), NE4049 (YU.v1), and NE4051 (YU.v2) (Table 1). Isolates NE4053 and NE5046 both had *ospC* group A1 and were therefore designated A1 variant 1 (A1.v1) and A1 variant 2 (A1.v2). Similarly, isolates NE4049 and NE4051 both had *ospC* group YU and were therefore designated YU variant 1 (YU.v1) and YU variant 2 (YU.v2). The *ospC* group of each isolate was determined via DNA sequencing (see below).

Table 1

The eight *Borrelia afzelii* isolates used in this study are shown. The isolates are differentiated according to their *ospC* gene group for which there are two different nomenclatures. For each isolate, the country and site of origin, the source of the isolate, and the Genbank accession number of the *ospC* gene sequence are shown.

Isolate	<i>ospC</i> Lagal ^a	<i>ospC</i> Bunikis ^b	Country	Site	Source	Genbank #
NE4053	A1.v1	A9.v1	Switzerland	Neuchâtel	Tick	JX103490
NE5046	A1.v2	A9.v2	Switzerland	Neuchâtel	Tick	JX103493
NE36	A2	A1	Switzerland	Thune	Mouse	KC207939
E61 ^c	A3	A3	Austria		Human	L42890
P/sto ^c	A4	A12	Germany		Human	AY150205
NE4054	ME	A2	Switzerland	Neuchâtel	Tick	JX103491
NE4049	YU.v1	A10.v1	Switzerland	Neuchâtel	Tick	JX103488
NE4051	YU.v2	A10.v2	Switzerland	Neuchâtel	Tick	JX103489

^a Nomenclature of *B. afzelii* *ospC* groups developed by Lagal et al. (2003) and by Pérez et al. (2011). This terminology is used in the present study.

^b Nomenclature of *B. afzelii* *ospC* groups developed by Bunikis et al. (2004).

^c Obtained from G. Baranton, Institute Pasteur, Paris.

Obtaining experimentally infected nymphal ticks

Experimentally infected nymphal ticks were used to measure tick-to-host transmission and were created as follows. Each of the eight *B. afzelii* isolates was cultured in 5 ml tubes containing BSK-H medium (Sinsky and Piesman, 1989) at 34 °C and spirochete densities were estimated using a Helber counting chamber. For each of the eight *B. afzelii* isolates, at least two naïve BALB/C mice were inoculated subcutaneously in the neck with 200 µl of BSK medium containing 2.0 × 10⁶ spirochetes/ml. Thirty days post-inoculation, mice were infested with approximately 100 *I. ricinus* larvae. Infested mice were placed in special cages to facilitate the collection of blood-engorged larvae. Blood-engorged larvae were placed in collecting tubes and were allowed to moult to the nymphal stage (Graf, 1978). One month after moulting, a haphazardly selected subset of 'sentinel' nymphs (*n*=13/mouse) was screened to determine the proportion of infected ticks for each isolate. For the eight isolates, the proportion of infected nymphs ranged from 0.154 (2/13) to 1.000 (13/13). The remaining nymphal ticks that had not been used for screening and that had fed on the same mice as the sentinel nymphs will be referred to as the 'challenge' nymphs. These challenge nymphs, for which the strain-specific probability of infection had been estimated from the sentinel nymphs, were used to challenge the mice and measure the strain-specific tick-to-host transmission rates (see below).

DNA extraction, amplification and detection of spirochetes

PCR was used to determine whether *I. ricinus* nymphs were infected with *B. afzelii*. DNA was extracted from ticks using the boiling ammonium hydroxide method described by Morán Cadenas et al. (2007a). The PCR targeted the variable region between the 23S and the 5S ribosomal gene following the protocol described by Morán Cadenas et al. (2007a). This gene is variable among *Borrelia* genospecies but not within a *Borrelia* genospecies (Morán Cadenas et al., 2007a). We therefore assumed that the sensitivity of our detection assay was the same for all eight *B. afzelii* isolates. To detect spirochete DNA, the amplicons from the PCR were used in a reverse line blot (RLB) assay that contained specific probes for *B. afzelii* (Morán Cadenas et al., 2007a).

Sequencing of the *ospC* gene

Part of the *ospC* gene from spirochete DNA extractions was amplified by PCR with primers ospC20 m (5'-AAT AAT TCA GGG AAA GGT GG-3') and ospC210 (modified from Earnhart et al., 2005). Spirochete DNA was obtained from culture thermolysates following the protocol described by Jouda et al. (2004). PCR reactions were then performed in a 50 µl volume containing 10 µl of the extracted DNA, 1× PCR buffer with 1.5 mM MgCl₂, 50 µM of each dNTP, 0.2 µM of each primer, 125 µM MgCl₂, and 1.5 units of Taq

polymerase. Reactions were performed under the following conditions: initial denaturation step at 94 °C for 180 seconds (s), 35 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, elongation at 72 °C for 60 s and final annealing step at 72 °C for 420 s. PCR products were purified using a commercial purification kit (QIAquick® PCR Purification Kit, QIAGEN). For each isolate, 1 to 3 amplicons were sent to Microsynth AG (Balgach, Switzerland) for sequencing.

Isolates of *B. burgdorferi* sensu lato (s. l.) often contain multiple *ospC* strains. As part of another study, we recently used 454-sequencing to test the purity of four isolates that were used in the present study. For isolates A2, A3, A4, and YU.v1 this approach obtained 873, 977, 1105, and 1313 sequences that were 99.20%, 100.0%, 99.70%, and 100.00% pure for *ospC* groups A2, A3, A4, and YU, respectively. This deep sequencing approach showed that these four isolates were dominated by a single *ospC* strain.

For each of the four remaining isolates, A1.v1, A1.v2, ME, YU.v2, we checked the purity by sequencing 20 clones containing the amplicons from a nested *ospC* PCR reaction following the protocol described by Bunikis et al. (2004). The cloning reactions were performed using the TOPO® TA cloning kit for sequencing with chemically competent cells (Invitrogen). This work showed that the four isolates were pure at this admittedly low level of resolution. For genetic material, we used the sentinel nymphs that tested positive for *B. afzelii* on the RLB. For isolate A1.v1, we were unable to amplify the *ospC* gene from four RLB-positive sentinel nymphs and we therefore used the culture thermolysate to determine strain purity and re-confirm the identity of the *ospC* gene. We then confirmed that the RLB-positive sentinel nymphs of isolate A1.v1 were infected with *B. afzelii* by amplifying and sequencing the *recA* gene as described in Richter et al. (2006). The presence of the *ospC* gene in the isolate but not the sentinel nymphs suggests that isolate A1.v1 lost the *ospC* plasmid during the transmission cycle from needle to mouse to sentinel nymph. In the results, we therefore excluded isolate A1.v1 from all of the statistical tests because the loss of the *ospC* plasmid was an artefact of culturing spirochetes.

Transmission dynamics experiments

Challenge of mice with *B. afzelii*-infected nymphs and tick-to-host transmission

To compare tick-to-host transmission among the eight *B. afzelii* isolates, four to ten pathogen-free mice were each infested with experimentally infected challenge nymphs. The protocol for infection by tick-bite followed Crippa et al. (2002). The proportion of infected sentinel nymphs for each isolate (range = 0.154–1.000) was used to calculate the number of challenge nymphs (20–3) to ensure that each mouse was infested with an average of three infected challenge nymphs. For example, if the proportion of infected sentinel nymphs for a given isolate was 0.5, each mouse was infected with 3/0.5 = 6 challenge nymphs. Experimentally infected nymphs

were placed in plastic capsules that had been attached to the shaved backs of the mice with wax (Mbrow et al., 1994). Capsules were checked on a daily basis to confirm that nymphs had actually attached and additional nymphs were added when this was not the case. Infection status of mice was subsequently determined using xenodiagnosis and ear biopsy (see below).

The nymphal infestation protocol was designed so that each mouse would be infested with an average of three infected challenge nymphs. By chance some mice may have only been infested with uninfected ticks. The number of challenge nymphs and the proportion of infected sentinel nymphs were used to calculate the probability that each mouse was challenged with at least one infected challenge nymph. The geometric mean probability that each mouse was challenged with at least one infected challenge nymph was 0.993 (range = 0.967–0.999). The experiment-wide probability that all 45 mice were challenged with at least one infected challenge nymph was 0.736. Thus we are relatively confident (73.6%) that all 45 mice were challenged with at least one infected challenge nymph and very confident (96.4%) that at least 44 of the 45 mice were truly challenged.

Co-feeding transmission and systemic transmission

To measure co-feeding transmission between experimentally infected challenge nymphs and uninfected larvae, ~100 pathogen-free *I. ricinus* larvae from our laboratory colony were added to the capsules two days after the infestation with the challenge nymphs. To measure systemic transmission between infected mice and uninfected larvae, mice were additionally infested with ~100 xenodiagnostic larvae 30 days after the nymphal challenge. All blood-engorged larvae were collected, allowed to moult into nymphs, and tested for spirochete infection as described above. For each mouse we used 13 haphazardly selected nymphs to measure co-feeding transmission and 13 haphazardly selected nymphs to measure systemic transmission.

Assessment of mouse infection status

Ear biopsy was used as a second, independent measure of mouse infection status. Ear biopsies were taken from anesthetized mice at day 30 post-infection using surgical scissors after cleaning the skin with 70% ethanol. Skin samples were placed into 5 ml BSK-H medium at 34°C to allow isolation of *B. afzelii*. Cultures were screened for the presence of spirochetes using dark-field microscopy each week for one month. Mice were considered as systemically infected if mouse-to-larva systemic transmission produced at least one infected nymph and/or if the ear biopsy tested positive for spirochetes.

Statistical methods

The level of statistical significance used in this study was 0.05.

Tick-to-host transmission

The isolate A1.v1 was excluded from the statistical analysis of tick-to-host transmission. Generalized linear models with binomial error functions were used to compare the tick-to-host transmission rate among the seven remaining *B. afzelii* isolates (A1.v2, A2, A3, A4, ME, YU.v1, YU.v2). We used the *glm()* function in R.

Systemic (host-to-tick) transmission

The isolate A1.v1 was excluded from the statistical analysis of systemic transmission. Generalized linear mixed effects models with binomial error functions were used to compare the systemic transmission rate among the seven *B. afzelii* isolates that had this

mode of transmission. We used the *glmer()* function in R. The analysis was done on the subset of mice ($n = 36$ mice) that produced at least one infected tick via systemic transmission or that had a spirochete-positive ear biopsy. Isolate and *ospC* group were treated as fixed factors whereas mouse identity was treated as a random factor.

Co-feeding (tick-to-tick) transmission

The isolate A1.v1 was excluded from the statistical analysis of co-feeding transmission. Generalized linear mixed effects models with binomial error functions were used to compare the co-feeding transmission rate among the five *B. afzelii* isolates (A1.v2, A2, ME, YU.v1, YU.v2) that had this mode of transmission. We used the *glmer()* function in R. The analysis was done on the subset of mice ($n = 25$ mice) that produced at least one co-infected tick. Isolate and *ospC* group were treated as fixed factors whereas mouse identity was treated as a random factor.

Correlations between tick-to-host, systemic and co-feeding transmission

We tested for correlations among the three transmission components: (1) tick-to-host transmission, (2) systemic transmission, and (3) co-feeding transmission, using isolate as the unit of replication. We calculated two sets of correlation coefficients, which differed in how the systemic and co-feeding transmission rates were calculated for the isolates. In the first set, the isolate-specific estimates of systemic and co-feeding transmission were calculated over all seven isolates and all 45 mice were included regardless of their infection status. In the second set, the isolate-specific estimates of systemic transmission were based on the subset of systemically infected mice ($n = 36$ mice). Similarly, in the second set, the isolate-specific estimates of co-feeding transmission were based on the subset of mice that produced at least one co-infected tick ($n = 25$ mice), which excluded two isolates (A3, A4).

*Estimation of reproductive number (R_0) for *B. afzelii* isolates*

Recent theoretical developments have allowed the estimation of the reproductive number (R_0) for complex disease systems like tick-borne infections (Hartemink et al., 2008). These so-called next generation matrices describe the pathogen life cycle by keeping track of infected ticks of different stages (larva, nymph, adult) and their ability to transition into the next stage (via survival, development, and reproduction). The largest eigenvalue of this matrix estimates R_0 , which determines whether the pathogen can invade the host population. If $R_0 > 1$, the pathogen invades the population whereas if $R_0 < 1$, the pathogen declines towards extinction. These standardized estimates of R_0 facilitate comparison between different tick-borne pathogens and the testing of epidemiological hypotheses. For example, recent studies using this approach have shown that co-feeding transmission and aggregation of ticks on hosts make a critical contribution to the epidemiology of tick-borne encephalitis (Harrison and Bennett, 2012; Harrison et al., 2011; Hartemink et al., 2008).

In the present study, we used the next generation matrix approach to estimate R_0 for each of the eight isolates of *B. afzelii* (including isolate A1.v1) using the equations in the Appendix of Harrison et al. (2011). For *I. ricinus*, we used all the parameters from Table 1 in Harrison et al. (2011). For *B. afzelii*, we used the systemic duration of infection ($i = 120$ days), and the efficiency of vertical transmission ($r_A = 0.1$) from Table 2 in Harrison et al. (2011). For each of the eight isolates, we set co-feeding transmission (θ), host-to-larva transmission (p_L), and nymph-to-host transmission (q_N) to the isolate-specific parameter estimates in

the present study (Table 2). The model by Harrison et al. (2011) contained stage-specific host-to-tick (p_L, p_N, p_A), and stage-specific tick-to-host transmission parameters (q_L, q_N, q_A) for all three tick stages (larva, nymph, adult). We assumed that $p_L = p_N = p_A$ and that $q_L = q_N = q_A$ for the eight isolates. We set the proportion of competent hosts (h_c) at 0.50 because blood meal analysis of questing *I. ricinus* in Switzerland has shown that 40% to 60% of ticks feed on spirochete-incompetent hosts such as artiodactyls and carnivores (Morán Cadenas et al., 2007b). In summary, we used the next generation matrix method to estimate R_0 for each of the eight isolates of *B. afzelii*. To obtain a single estimate of R_0 for *ospC* strain YU, we took the geometric mean of the R_0 estimates of the two variants (YU.v1 and YU.v2). As a point of comparison, we calculated R_0 for *B. burgdorferi* s. l. using the parameters in Harrison et al. (2011): ($\theta = 0.56, p_L = p_N = p_A = 0.5, q_L = q_N = q_A = 0.8$).

Correlation between R_0 and the frequency of the *B. afzelii* *ospC* strains

The R_0 value of each *B. afzelii* *ospC* strain should determine its frequency in nature. A recent study by Pérez et al. (2011) estimated the frequency of ten *B. afzelii* *ospC* strains, of which six occurred in the present study (A1–A4, ME, and YU). Thus the study by Pérez et al. (2011) allowed us to test whether the R_0 value of each *B. afzelii* *ospC* strain measured in laboratory mice is predictive of its frequency in nature. The authors sampled ticks from two sites in Switzerland: Portes-Rouges (PR), Neuchâtel, Canton Neuchâtel and Staatswald (SW), Ins, Canton Bern. Isolates of *B. afzelii* were cultured from two distinct sources of ticks: questing nymphs and ticks that had been removed from rodents. For the PR site, 81 isolates from questing ticks and 89 isolates from rodent-derived ticks were genotyped. For the SW site, 51 isolates from questing ticks and 171 isolates from rodent-derived ticks were genotyped. The authors used cold single-strand conformational polymorphism analysis to identify the *ospC* group of each isolate. We used Pearson's correlation to test whether there was a significant correlation between the R_0 value and the frequency of the six *B. afzelii* *ospC* strains in the questing ticks and the rodent-derived ticks for the two sites combined and for each site separately.

Results

Correlation between xenodiagnosis and ear biopsy detection methods

The infection status of the mice was determined using two different methods: (1) PCR of xenodiagnostic ticks and (2) microscope

screening of ear biopsies cultured in BSK media. Of the 52 mice (including the seven mice for isolate A1.v1), 37 tested positive for infection with *B. afzelii* according to the xenodiagnosis assay and 30 tested positive according to the ear biopsy. Thus xenodiagnosis (37/52) was more sensitive than the ear biopsy (30/52) but this difference was not statistically significant (proportion test, $p = 0.219$). Of the 30 mice that tested positive for the ear biopsy, 29 also tested positive for the PCR. Thus there was a strong correlation in infection status between the two methods ($r = 0.658, t = 6.17, df = 50, p < 0.0001$). The strong correlation between the two detection methods shows that our PCR results were not biased by differential sensitivity between strains. The only mouse that tested positive via ear biopsy but not xenodiagnoses had been challenged with isolate A1.v1.

Variation in tick-to-host transmission among *B. afzelii* isolates

All seven *B. afzelii* isolates succeeded in infecting mice via tick bite. The average tick-to-host transmission rate was 82.2% (37 infected mice/45 challenged mice) over the seven isolates (Table 2). The tick-to-host transmission rate of strain ME (40.0% = 4/10) was lower than the other six isolates combined (94.3% = 33/35 mice) and this difference was statistically significant ($\chi^2 = 13.33, df = 1, p < 0.0003$).

Variation in systemic (host-to-larva) transmission among *B. afzelii* isolates

All seven *B. afzelii* isolates had systemic transmission (Fig. 1; Table 2). Systemic transmission occurred in 80.0% (36/45) of the mice and 65.5% (305/466) of the xenodiagnostic ticks from these mice were infected. For the subset of systemically infected mice ($n = 36$), there were significant differences in systemic transmission among the seven isolates ($\chi^2 = 32.86, df = 6, p < 0.0001$), among the six *ospC* strains ($\chi^2 = 26.92, df = 5, p < 0.0001$), and between variants belonging to the same *ospC* strain ($\chi^2 = 5.94, df = 1, p = 0.015$). Post hoc model simplification allowed us to combine the seven isolates with systemic transmission into low (A4, ME; range = 15.4–30.8%), medium (A2, A3, YU.v2; range = 53.8–70.4%), and high (A1.v2, YU.v1; range = 85.5–92.3%) transmission groups. The medium transmission group was significantly different from the low ($\chi^2 = 19.66, df = 5, p < 0.002$) and the high transmission group ($\chi^2 = 13.41, df = 5, p = 0.020$).

Table 2

The three transmission components are shown for the eight *B. afzelii* isolates. The proportion of infected mice is the tick-to-host transmission rate. An infected mouse was defined as having systemic transmission and/or a spirochete-positive ear biopsy. Co-feeding mice refers to the proportion of mice that produced at least one infected tick via co-feeding. The systemic transmission rates show the proportion of ticks that acquired spirochetes from the subset of 36 systemically infected mice. The co-feeding transmission rates show the proportion of ticks that acquired spirochetes via co-feeding from the subset of 25 mice with at least one co-feeding event.

<i>ospC</i> (Lagal)	<i>B. afzelii</i> isolate	Infected mice	Co-feeding mice	Systemic transmission	Co-feeding transmission
<i>B. b. s. l.</i>		0.80 ^a		0.50 ^a	0.56 ^a
A1.v1 ^b	NE4053	2/7 (28.6)	1/7 (14.3)	1/26 (3.8)	2/13 (15.4)
A1.v2 ^b	NE5046	4/4 (100.0)	4/4 (100.0)	48/52 (92.3)	17/52 (32.7)
A2	NE36	4/4 (100.0)	2/4 (50.0)	35/52 (67.3)	8/26 (30.8)
A3	E61	3/4 (75.0)	0/4 (0.0)	21/39 (53.8)	0/52 ^c (0.0)
A4	P/sto	4/4 (100.0)	0/4 (0.0)	8/52 (15.4)	0/52 ^c (0.0)
ME	NE4054	4/10 (40.0)	1/10 (10.0)	12/39 (30.8)	1/26 (3.8)
YU.v1 ^b	NE4049	8/9 (88.9)	8/9 (88.9)	100/117 (85.5)	129/195 (66.2)
YU.v2 ^b	NE4051	9/10 (90.0)	10/10 (100.0)	81/115 (70.4)	103/221 (46.6)
Total		36/45 (80.0)	25/45 (55.6)	305/466 (65.5)	258/520 (49.6)

^a Parameter estimates of *Borrelia burgdorferi* sensu lato (*B. b. s. l.*) were taken from Table 2 in Harrison et al. (2011).

^b The suffixes v1 and v2 refer to variant 1 and variant 2.

^c These nymphs were not included in the calculation of the total co-feeding transmission rate (258/520 = 49.6%).

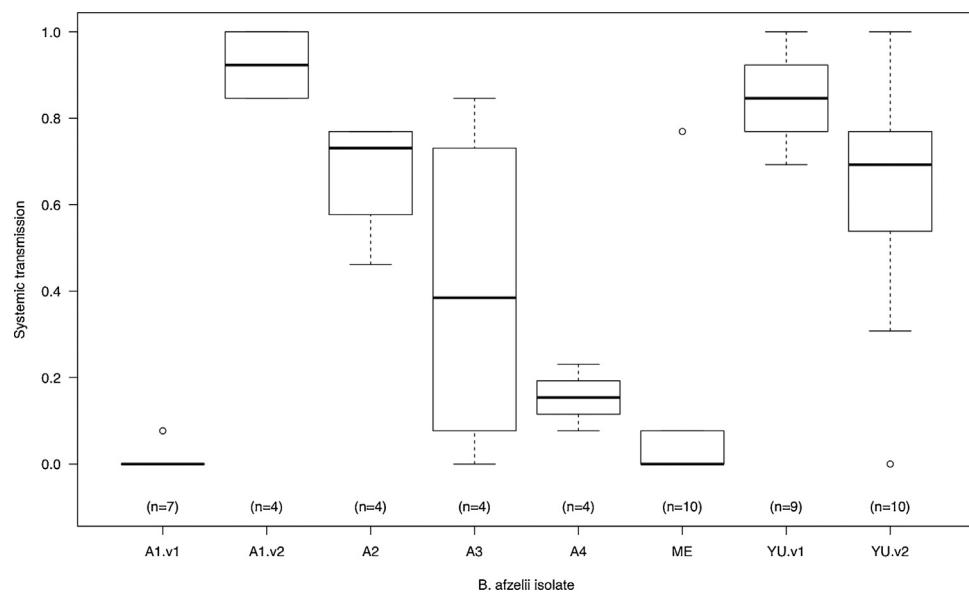


Fig. 1. Systemic transmission differs among the eight isolates of *B. afzelii*. Each data point represents the systemic transmission rate for one mouse and is based on 13 ticks. The numbers of mice (*n*) used for each isolate are shown in brackets above the x-axis. Shown are the median (black line), 25th and 75th percentiles (edges of the box), the minimum and maximum values (whiskers), and outliers (circles).

Variation in co-feeding (nymph-to-larva) transmission among *B. afzelii* isolates

Three isolates (A3, A4, ME) had little or no co-feeding transmission, two isolates (A1.v2, A2) had intermediate co-feeding transmission, and two isolates (YU.v1, YU.v2) had high co-feeding transmission (Fig. 2; Table 2). Co-feeding transmission occurred on 55.6% (25/45) of the mice and 49.6% (258/520) of the larvae on those mice were infected via this mode of transmission (Table 2). There were 11 mice that had systemic transmission but no co-feeding transmission. Interestingly, one mouse infected with isolate YU.v2 had co-feeding transmission (46.2% = 6/13) but no systemic transmission (0.0% = 0/13). For the subset of mice with co-feeding transmission (*n* = 25 mice), there were significant differences

in co-feeding transmission among the five isolates ($\chi^2 = 10.23$, *df* = 4, *p* = 0.037) and among the four *ospC* strains ($\chi^2 = 7.95$, *df* = 3, *p* = 0.047) but no differences between variants belonging to the same *ospC* strain ($\chi^2 = 2.28$, *df* = 1, *p* = 0.131). Post hoc model simplification allowed us to combine the five isolates with co-feeding transmission into low (ME; mean = 3.8%), medium (A1.v2, A2; range = 30.8–32.7%), and high (YU.v1, YU.v2; range = 46.6–66.2%) transmission groups. The medium transmission group was not significantly different from either the low ($\chi^2 = 4.65$, *df* = 3, *p* = 0.200) or the high transmission group ($\chi^2 = 5.94$, *df* = 3, *p* = 0.115). The difference between the low and high transmission group was most pronounced when the medium group was combined with the low transmission group (A1, A2, ME versus YU; $\chi^2 = 5.58$, *df* = 1, *p* = 0.018).

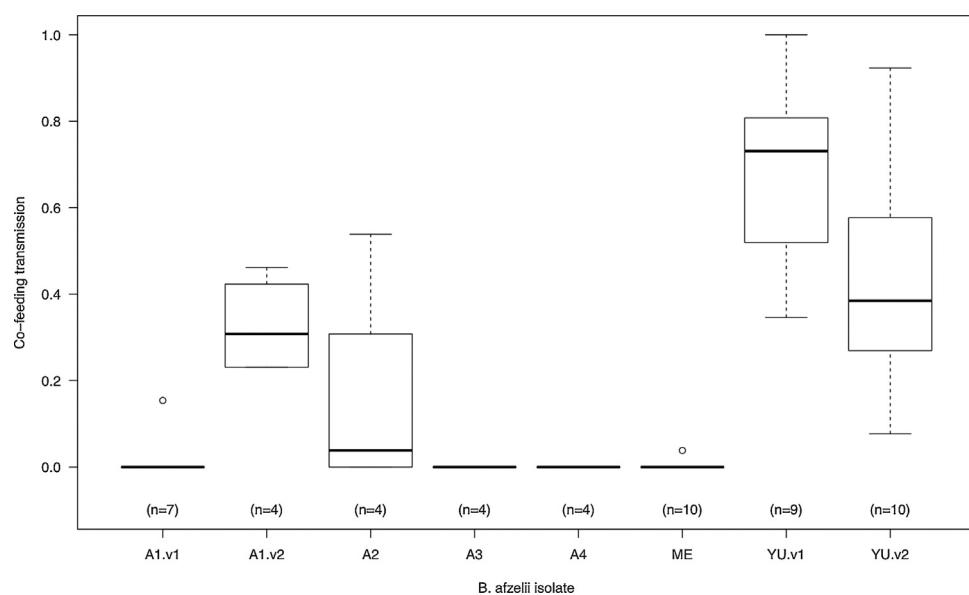


Fig. 2. Co-feeding transmission differs among the eight isolates of *B. afzelii*. Each data point represents the co-feeding transmission rate for one mouse and is based on 13–26 ticks. The numbers of mice (*n*) used for each isolate are shown in brackets above the x-axis. Shown are the median (black line), 25th and 75th percentiles (edges of the box), the minimum and maximum values (whiskers), and outliers (circles).

Table 3

Correlations between the three transmission components are shown. The first set of correlations was based on all eight isolates and all 45 mice. In the second set of correlations, the strain-specific estimates of systemic and co-feeding transmission were based on a subset of mice ($n=36$ and 25, respectively), which resulted in the exclusion of some strains. Shown are the Pearson's correlation coefficient (r), the t -statistic (t), the degrees of freedom (df), and the p -value (p).

Trait 1	Trait 2	Isolates	Mice	r	t	df	p
Tick-to-host	Systemic	7	45	0.651	1.916	5	0.113
Tick-to-host	Co-feeding	7	45	0.530	1.399	5	0.221
Systemic	Co-feeding	7	45	0.793	2.916	5	0.033
Tick-to-host	Systemic	7	36	0.474	1.2043	5	0.282
Tick-to-host	Co-feeding	5	25	0.786	2.2017	3	0.115
Systemic	Co-feeding	5	25	0.760	2.0251	3	0.136

Correlations between tick-to-host, systemic, and co-feeding transmission

The correlations between the three transmission components were all positive (Table 3). When all the seven *B. afzelii* isolates and all the 45 mice were included, the correlation between systemic and co-feeding transmission was statistically significant ($r=0.793$, $p=0.033$) but the correlations between tick-to-host transmission and systemic transmission ($r=0.651$, $p=0.113$) and between tick-to-host transmission and co-feeding transmission ($r=0.530$, $p=0.221$) were not significant. When the isolate-specific estimates of systemic and co-feeding transmission were restricted to the subset of systemically infected mice ($n=36$) and the subset of mice that had at least one co-feeding event ($n=25$), the correlations remained positive but were not significant (Table 3). In general, strains that were highly infectious for mice had high systemic and co-feeding transmission. Conversely, strains that were less infectious for mice had lower systemic and co-feeding transmission.

The reproductive number (R_0) of the *B. afzelii* isolates

The reproductive number (R_0) was greater than 2.0 for all *B. afzelii* isolates except A1.v1 ($R_0=0.878$; supplementary material, Table S1). Thus seven isolates of *B. afzelii* were capable of invading and persisting in a Lyme borreliosis system where half of the hosts ($h_c=0.50$) have a reservoir competence similar to the laboratory mice in this study. Of the seven isolates capable of invasion and persistence, R_0 ranged from 2.036 (strain ME) to 5.519 (strain YU.v1) (supplementary material, Table S1). As a point of comparison, the value of R_0 for *B. burgdorferi* s. l. was 4.052 using the parameter estimates from Table 2 in Harrison et al. (2011).

Correlation between R_0 and the frequency of the *B. afzelii* ospC groups

We tested whether the reproductive number (R_0) of each *B. afzelii* ospC strain measured in laboratory mice was predictive of its frequency in nature using data from a study by Pérez et al. (2011). The correlation between R_0 and the frequency in the questing nymphs (for the two sites combined) of the six *B. afzelii* ospC strains was positive but not statistically significant ($r=0.670$, $t=1.80$, df = 4, $p=0.146$; Fig. 3; Table 4). Despite the lack of statistical significance, it is worth pointing out that the R_0 values from a laboratory study accounted for 44.9% of the variation in the frequencies of the six *B. afzelii* ospC strains in questing nymphs in the field (Fig. 3). The results were similar when the correlation test was conducted separately for each site (Table 4). Finally, none of the correlations between R_0 and the ospC strain frequencies in the rodent-derived nymphs were significant (Table 4).

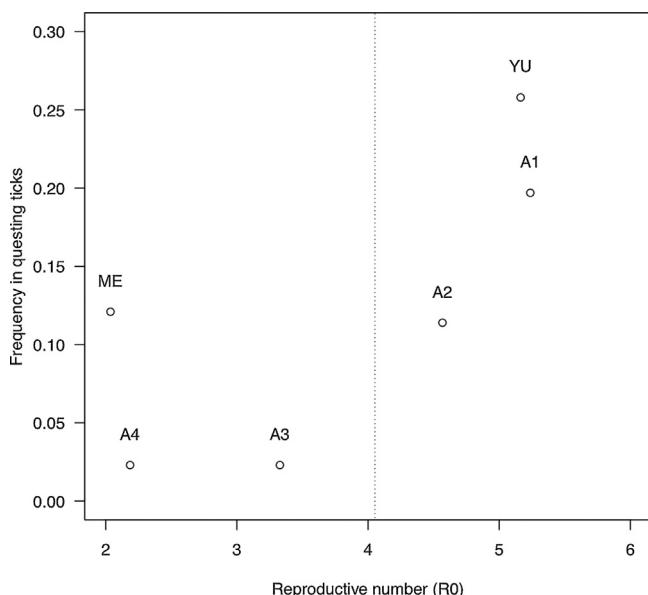


Fig. 3. The relationship between the reproductive number (R_0) and the frequency of the six *B. afzelii* ospC strains in questing ticks is shown. The R_0 value for YU was calculated as the geometric mean of YU.v1 and YU.v2. The stippled line is an estimate of R_0 for *B. burgdorferi* sensu lato from the literature.

Table 4

The correlation between the log-transformed reproductive number (R_0) of the *B. afzelii* ospC strains and the strain-specific frequencies in the field are shown. The frequencies of the ospC strains in the field were calculated either from the questing nymphs (F_{questing}) or the rodent-derived nymphs (F_{rodent}), respectively. The correlations are shown separately for the Portes-Rouges site (PR), the Staatswald site (SW), and the two sites combined (Comb). Shown are Pearson's correlation coefficient (r), the t -statistic (t), the degrees of freedom (df), and the p -value (p).

Frequency	Site	r	t	df	p
F_{questing}	PR	0.426	0.941	4	0.400
F_{questing}	SW	0.768	2.401	4	0.074
F_{questing}	Comb	0.670	1.804	4	0.146
F_{rodent}	PR	0.198	0.405	4	0.706
F_{rodent}	SW	0.786	2.545	4	0.064
F_{rodent}	Comb	0.660	1.759	4	0.153

Discussion

The most interesting result from this study was the observation that *B. afzelii* populations contain some strains that are highly successful at all three transmission components of the Lyme borreliosis cycle. Strains YU.v1, YU.v2, A1.v2, and A2 all had high rates of tick-to-host, systemic (Fig. 1), and co-feeding transmission (Fig. 2), and consequently these four isolates all had an R_0 value greater than 4.0 (Fig. 3). In contrast, *B. afzelii* strains A3, A4, and ME were less successful at all three components of transmission and consequently these three isolates had an R_0 less than 4.0. With respect to the trade-off hypothesis, the correlation coefficients of the three pairs of transmission components were always positive (Table 3). Thus in contrast to our prediction that *B. afzelii* strains would be specialized on different aspects of the life cycle, we found no evidence of trade-offs among the three canonical transmission components.

In the absence of competition between pathogen strains, theory predicts that the reproductive number (R_0) of each strain will determine its prevalence in the pathogen population (Gupta et al., 1998). Assuming that performance in BALB/c mice is correlated with performance in wild rodents, we expected a positive correlation between the R_0 values of the *B. afzelii* strains in laboratory mice and their frequencies in questing ticks as measured in a recent

field study (Pérez et al., 2011). The R_0 values estimated for the six *B. afzelii* *ospC* strains in the present study explained 45% of the variation in the strain-specific frequencies in the field study by Pérez et al. (2011) (Fig. 3). Despite the high r^2 value, the positive relationship between R_0 and strain frequency in the field was not statistically significant because of the small sample size (Table 4). However, the relationship between R_0 and the strain-specific frequency provides a useful framework for investigating the ecology of the different *B. afzelii* *ospC* strains. Strain YU had the highest R_0 value and the highest prevalence whereas other strains such as A2 and A3 had high R_0 values that were not commensurate with their low frequencies in the field (Fig. 3). These strains suggest that we are missing other important ecological factors that determine strain performance in nature. One obvious explanation is that high performance in laboratory mice may not translate to the relevant reservoir hosts in the field. An experimental infection study on two strains of *B. burgdorferi* s. s. found that host-to-tick transmission was considerably lower in natural rodent reservoir hosts than in laboratory mice (Hanincova et al., 2008). According to the multiple niche hypothesis, the different *ospC* strains of *B. burgdorferi* s. s. are associated with different mammalian reservoir hosts (Brisson and Dykhuizen, 2004). This explanation may apply to strain ME, which had the lowest fitness in laboratory mice ($R_0 = 2.036$) but which reached the third highest frequency in questing ticks (0.121) at the two sites combined (Fig. 3; supplementary material, Table S1). Alternative reservoir hosts for *B. afzelii* in Switzerland include birds, wild boar, and small carnivores, as shown by host blood meal analysis of questing nymphs (Morán Cadenas et al., 2007b).

Our study greatly condensed the tick component of the spirochete life cycle. Thus another explanation for the low field prevalence of some strains is that they perform poorly in the tick vector. In nature, *Borrelia* spends most of the year, including the winter, inside the nymphal tick. By contrast, in the present study, the spirochetes spent less than two months inside the ticks before infecting the mice, and the ticks were kept at room temperature. This explanation may be appropriate for strain A3, which had a low frequency in questing ticks (0.023) at the two sites combined in the study by Pérez et al. (2011). In contrast, strain A2 had an appreciable frequency in questing nymphs (0.114) at the two sites combined, suggesting that this strain does not suffer from poor survival in overwintering nymphs.

Competition among strains in multiply infected hosts or ticks is another factor that can influence the strain-specific frequencies in the field. Previous work has shown that infections with multiple *Borrelia* *ospC* strains are common in both the tick vector (Pérez et al., 2011; Qiu et al., 2002; Wang et al., 1999) and the rodent reservoir (Andersson et al., 2013; Brisson and Dykhuizen, 2004; Pérez et al., 2011). We used single-strain infections in the present study and so we did not consider how competitive interactions between strains might affect R_0 . We have found substantial competition among *Borrelia* genospecies inside the tick (Herrmann et al., 2013) and similar phenomena might occur among *Borrelia* *ospC* strains. With respect to the vertebrate host, there is some evidence for *B. burgdorferi* s. s. that multiple infections change the temporal dynamics of systemic transmission (Derdakova et al., 2004). A recent field study on multiple infections of *B. afzelii* in wild rodents found that strains with genetically similar *OspC* antigens were less likely to co-occur in the same host than *ospC* strains that were genetically different (Andersson et al., 2013). This study suggests that a given *OspC* antigen induces an antibody response that reduces the probability of tick-to-host transmission of closely related *ospC* strains. Thus cross-reactive antibodies in the vertebrate host can play an important role in mediating indirect competition between strains of *Borrelia*.

There was significant genetic variation among the *B. afzelii* *ospC* strains in all three components of transmission: tick-to-host, systemic, and co-feeding transmission. To date, most studies

investigating fitness differences among strains of *Borrelia* only measure systemic transmission (Derdakova et al., 2004; Hanincova et al., 2008). Other studies comparing strains of *B. burgdorferi* s. s. in laboratory mice have investigated the relationship between spirochete load in mouse tissues and measures of pathology such as arthritis, carditis, and ankle swelling (Dolan et al., 2004; Wang et al., 2001, 2002; Zeidner et al., 2001). These studies often find a positive relationship between spirochete load, ability to disseminate to different tissues, and pathology, and strains that score high on all these phenotypes are often labelled as invasive (Dolan et al., 2004; Wang et al., 2001, 2002; Zeidner et al., 2001). However, as none of these studies measured tick-to-host or host-to-tick transmission, the relationships between spirochete load, pathology, and *Borrelia* fitness (R_0) remain unknown.

The mechanisms that determine transmission success in *Borrelia* pathogens are probably related to the spirochete loads inside the host tissues and the tick salivary glands. A recent study on wild rodents found that the load of *B. afzelii* spirochetes inside host tissues determined the probability of systemic transmission but not the spirochete load inside these same ticks after they had moulted into nymphs (Raberg, 2012). A study on *B. burgdorferi* s. s. found that strains that are common in questing ticks have higher spirochete loads when inoculated into their natural rodent hosts than strains that are rare in questing ticks (Baum et al., 2012). Thus we expect the *B. afzelii* strains with high values of R_0 to have higher spirochete loads in their rodent reservoir hosts. Genetic variation in tick-to-host transmission may likewise depend on the density of spirochetes inside the tick salivary glands. During the tick blood meal, spirochetes migrate from the tick midgut to the salivary glands from where they are injected into the feeding lesion (de Silva and Fikrig, 1995; Gern et al., 1990, 1996; Ohnishi et al., 2001; Piesman et al., 2001). Thus genetic variation in tick-to-host transmission may depend on the speed at which the spirochetes colonize the tick salivary glands following attachment to the vertebrate host (Crippa et al., 2002).

Another important result from this study was the first demonstration of genetic variation in co-feeding transmission in a tick-borne pathogen. To date, all theoretical studies investigating R_0 of *B. burgdorferi* s. l. assume that the co-feeding transmission rate is 56% (Harrison and Bennett, 2012; Harrison et al., 2011; Hartemink et al., 2008). The present study found that co-feeding transmission rates range from 0.0% to 66.2% among strains of *B. afzelii* (Table 2). The existence of genetic variation for co-feeding transmission implies that this trait can evolve by natural selection. While co-feeding is the main mode of transmission for tick-borne viruses (Randolph, 2011; Randolph et al., 1996), its importance for *Borrelia* pathogens is controversial (Richter et al., 2002; Voordouw, 2015). Co-feeding transmission is believed to be less important to *Borrelia* pathogens because systemic infections can be so long-lived in the rodent reservoir hosts (Gern et al., 1994; Humair et al., 1999). Recent theoretical work has shown that co-feeding transmission makes a modest contribution to the fitness of *Borrelia* pathogens and is not necessary for invading naive tick populations (Harrison et al., 2011; Hartemink et al., 2008). However, these models ignore the fact that multiple infections are ubiquitous in Lyme borreliosis systems and that strains of *Borrelia* may be in constant competition with each other. Under these circumstances, the modest contribution of co-feeding transmission might provide a decisive fitness advantage. In the present study, the four strains with the highest values of R_0 (A1.v2, A2, YU.v1, YU.v2) also had the highest values of co-feeding transmission (32.7%, 30.8%, 46.6% and 62.2%). In contrast, the three strains with the lowest values of R_0 (A3, A4, ME) had very little co-feeding transmission (<4.0%). In summary, the present study found substantial variation in co-feeding transmission among strains of *B. afzelii* and that this mode of transmission is associated with high values of R_0 .

The field study by Pérez et al. (2011) suggested that *B. afzelii* ospC strain YU was highly efficient at co-feeding transmission and this observation was one of the motivating factors for the present study. A critical condition for co-feeding transmission is coincident feeding of nymphal and larval ticks on the same rodent reservoir host (Randolph et al., 1999). Pérez et al. (2011) compared the community of *B. afzelii* ospC strains between two different field sites where the wild rodent populations differed in the frequency of coincident feeding and hence the potential for co-feeding transmission. Their study found that *B. afzelii* ospC strain YU was much more common in the site with high potential for co-feeding transmission (Pérez et al., 2011). The present study confirms that ospC strain YU is indeed highly efficient at co-feeding transmission.

The OspC protein plays a critical role in the tick-to-host transmission step of *B. burgdorferi* s. l. pathogens (Fingerle et al., 2007; Grimm et al., 2004; Pal et al., 2004; Tilly et al., 2006). In the present study, the importance of the ospC gene was shown by the difference in fitness between the two variants of the A1 strain. Variant A1.v1 had lost its ospC gene and was much less efficient at infecting mice than variant A1.v2 (Table 2). Interestingly, variant A1.v1 maintained some co-feeding transmission (Table 2). The OspC protein allows the spirochetes to disseminate from the tick bite and establish a systemic infection inside the vertebrate host (Grimm et al., 2004; Tilly et al., 2006). As tick-to-tick transmission occurs before systemic infection, the co-feeding transmission success of the A1.v1 variant was consistent with the functional role of the OspC protein (Radolf and Caimano, 2008).

Due to its role in host invasion, the ospC gene has received much interest from a public health perspective. Studies on *B. burgdorferi* s. s. (Seinost et al., 1999) and *B. afzelii* (Baranton et al., 2001) have shown that only a limited set of ospC groups are associated with disseminated infections in humans, although this concept is not without controversy (Alghaferi et al., 2005; Earnhart et al., 2005; Lagal et al., 2003). Studies using more sophisticated measures of invasiveness have largely confirmed that the ospC locus remains a useful marker for studying the human pathogenicity of *Borrelia* strains (Dykhuisen et al., 2008; Wormser et al., 2008). The present study found that *B. afzelii* ospC strains A1, A2, A3, A4, and YU were highly infectious to laboratory mice when delivered via tick bite. Two of these three ospC strains (A1 and A4) are known to cause disseminated infections in humans (Baranton et al., 2001; Lagal et al., 2003).

In summary, the present study found genetic variation among *B. afzelii* strains in all three components of transmission. We found positive correlations between tick-to-host transmission, systemic transmission, and co-feeding transmission and thus no evidence of trade-offs between these pathogen life history traits. Certain strains of *B. afzelii* were successful in all three components of transmission and consequently these strains had high values of R_0 . Our laboratory estimates of R_0 explained an important (but not statistically significant) percentage of the variation in the strain-specific frequencies measured in another field study. The present study shows the value of estimating R_0 in a well-chosen animal model to understand the strain composition of a pathogen population in the field.

Author's contributions

LG conceived and designed the study. NT and SM conducted the experimental work. The sequencing work to determine the purity of the isolates was done by JD. MJV conducted the statistical analyses and the next generation matrix models. MJV and NT wrote the manuscript. LG and JD edited the manuscript.

Competing interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by two Swiss National Science Foundation (SNSF) grants to Lise Gern (FN 320030_113936 and FN 310030_127064) and one SNSF grant to Maarten Voordouw (FN 31003A_141153). The University of Neuchâtel supported this work by an SNSF overhead grant to Maarten Voordouw (U.01851.01 project 4.5). Thanks to Guy Baranton for the *B. afzelii* isolates E61 and P/sto. Thanks to Dolores Genné for help with cloning and sequencing. The members of the working group 'Tiques et Maladies à Tiques' (GDR REID) provided insightful discussions. Thanks to Jacob Koella and three anonymous reviewers for comments on this manuscript. This study is part of the PhD thesis of Nicolas Tonetti.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2015.02.007.

References

- Alghaferi, M.Y., Anderson, J.M., Park, J., Auwaerter, P.G., Aucot, J.N., Norris, D.E., Dumler, J.S., 2005. *Borrelia burgdorferi* OspC heterogeneity among human and murine isolates from a defined region of northern Maryland and southern Pennsylvania: lack of correlation with invasive and noninvasive genotypes. *J. Clin. Microbiol.* 43, 1874–1879.
- Andersson, M., Scherman, K., Raberg, L., Råberg, L., 2013. Multiple-strain infections of *Borrelia afzelii*: a role for within-host interactions in the maintenance of antigenic diversity? *Am. Nat.* 181, 545–554.
- Baranton, G., Seinost, G., Theodore, G., Postic, D., Dykhuizen, D., 2001. Distinct levels of genetic diversity of *Borrelia burgdorferi* are associated with different aspects of pathogenicity. *Res. Microbiol.* 152, 149–156.
- Baum, E., Hue, F., Barbour, A.G., 2012. Experimental infections of the reservoir species *Peromyscus leucopus* with diverse strains of *Borrelia burgdorferi*, a Lyme disease agent. *mBio* 3, e00434–512.
- Brisson, D., Dykhuizen, D.E., 2004. ospC diversity in *Borrelia burgdorferi*: different hosts are different niches. *Genetics* 168, 713–722.
- Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., Barbour, A.G., 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology* 150, 1741–1755.
- Crippa, M., Rais, O., Gern, L., 2002. Investigations on the mode and dynamics of transmission and infectivity of *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* in *Ixodes ricinus* ticks. *Vector Borne Zoonot. Dis.* 2, 3–9.
- de Roode, J.C., Pansini, R., Cheesman, S.J., Helinski, M.E.H., Huijben, S., Wargo, A.R., Bell, A.S., Chan, B.H.K., Walliker, D., Read, A.F., 2005. Virulence and competitive ability in genetically diverse malaria infections. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7624–7628.
- de Roode, J.C., Yates, A.J., Altizer, S., 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7489–7494.
- de Silva, A.M., Fikrig, E., 1995. Growth and migration of *Borrelia burgdorferi* in *Ixodes* ticks during blood feeding. *Am. J. Trop. Med. Hyg.* 53, 397–404.
- Derdakova, M., Dudioak, V., Brei, B., Brownstein, J.S., Schwartz, I., Fish, D., 2004. Interaction and transmission of two *Borrelia burgdorferi* sensu stricto strains in a tick-rodent maintenance system. *Appl. Environ. Microbiol.* 70, 6783–6788.
- Dolan, M., Piesman, J., Schneider, B., Schriefer, M., Brandt, K., Zeidner, N., 2004. Comparison of disseminated and nondisseminated strains of *Borrelia burgdorferi* sensu stricto in mice naturally infected by tick bite. *Infect. Immun.* 72, 5262–5266.
- Dykhuizen, D.E., Baranton, G., 2001. The implications of a low rate of horizontal transfer in *Borrelia*. *Trends Microbiol.* 9, 344–350.
- Dykhuizen, D.E., Brisson, D., Sandigursky, S., Wormser, G.P., Nowakowski, J., Nadelman, R.B., Schwartz, I., 2008. Short report: The propensity of different *Borrelia burgdorferi* sensu stricto genotypes to cause disseminated infections in humans. *Am. J. Trop. Med. Hyg.* 78, 806–810.
- Earnhart, C.G., Buckles, E.L., Dumler, J.S., Marconi, R.T., 2005. Demonstration of OspC type diversity in invasive human Lyme disease isolates and identification of previously uncharacterized epitopes that define the specificity of the OspC murine antibody response. *Infect. Immun.* 73, 7869–7877.
- Ebert, D., 1998. Evolution – experimental evolution of parasites. *Science* 282, 1432–1435.
- Ebert, D., Bull, J.J., 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.* 11, 15–20.

- Fingerle, V., Goettner, G., Gern, L., Wilske, B., Schulte-Spechtel, U., 2007. *Complementation of a Borrelia afzelii OspC mutant highlights the crucial role of OspC for dissemination of Borrelia afzelii in Ixodes ricinus*. Int. J. Med. Microbiol. 297, 97–107.
- Fraser, C., Hollingsworth, T.D., Chapman, R., de Wolf, F., Hanage, W.P., 2007. Variation in HIV-1 set-point viral load: Epidemiological analysis and an evolutionary hypothesis. Proc. Natl. Acad. Sci. U. S. A. 104, 17441–17446.
- Gern, L., Lebet, N., Moret, J., 1996. Dynamics of *Borrelia burgdorferi* infection in nymphal *Ixodes ricinus* ticks during feeding. Exp. Appl. Acarol. 20, 649–658.
- Gern, L., Rais, O., 1996. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acar: Ixodidae). J. Med. Entomol. 33, 189–192.
- Gern, L., Siegenthaler, M., Hu, C.M., Leuba-Garcia, S., Humair, P.F., Moret, J., 1994. *Borrelia burgdorferi* in rodents (*Apodemus flavicollis* and *A. sylvaticus*): duration and enhancement of infectivity for *Ixodes ricinus* ticks. Eur. J. Epidemiol. 10, 75–80.
- Gern, L., Zhu, Z., Aeschlimann, A., 1990. Development of *Borrelia burgdorferi* in *Ixodes ricinus* females during blood feeding. Ann. Parasitol. Hum. Comp. 65, 89–93.
- Graf, J.-F., 1978. Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae) – 1^{re} partie. Bull. Soc. Entomol. Suisse 51, 89–97.
- Grimm, D., Tilly, K., Byram, R., Steward, P.E., Krum, J.G., Bueschel, D.M., Schwan, T.G., Pollicastro, P.F., Elias, A.F., Rosa, P.A., 2004. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc. Natl. Acad. Sci. U. S. A. 101, 3142–3147.
- Gupta, S., Ferguson, N., Anderson, R., 1998. Chaos, persistence, and evolution of strain structure in antigenically diverse infectious agents. Science 280, 912–915.
- Hanincova, K., Ogden, N.H., Diuk-Wasser, M., Pappas, C.J., Iyer, R., Fish, D., Schwartz, I., Kurtenbach, K., 2008. Fitness variation of *Borrelia burgdorferi* sensu stricto strains in mice. Appl. Environ. Microbiol. 74, 153–157.
- Harrison, A., Bennett, N., 2012. The importance of the aggregation of ticks on small mammal hosts for the establishment and persistence of tick-borne pathogens: an investigation using the R(0) model. Parasitology 139, 1605–1613.
- Harrison, A., Montgomery, W.I., Bown, K.J., 2011. Investigating the persistence of tick-borne pathogens via the R-0 model. Parasitology 138, 896–905.
- Hartemink, N.A., Randolph, S.E., Davis, S.A., Heesterbeek, J.A.P., 2008. The basic reproduction number for complex disease systems: Defining R-0 for tick-borne infections. Am. Nat. 171, 743–754.
- Hellgren, O., Andersson, M., Raberg, L., Råberg, L., 2011. The genetic structure of *Borrelia afzelii* varies with geographic but not ecological sampling scale. J. Evol. Biol. 24, 159–167.
- Herrmann, C., Gern, L., Voordouw, M., 2013. Species co-occurrence patterns among Lyme borreliosis pathogens in the tick vector *Ixodes ricinus*. Appl. Environ. Microbiol. 79, 7273–7280.
- Hu, C.M., Cheminade, Y., Perret, J.L., Weynants, V., Lobet, Y., Gern, L., 2003. Early detection of *Borrelia burgdorferi* sensu lato infection in Balb/c mice by co-feeding *Ixodes ricinus* ticks. Int. J. Med. Microbiol. 293, 421–426.
- Humair, P.F., Gern, L., 1998. Relationship between *Borrelia burgdorferi* sensu lato species, red squirrels (*Sciurus vulgaris*) and *Ixodes ricinus* in enzootic areas in Switzerland. Acta Trop. 69, 213–227.
- Humair, P.F., Péter, O., Wallich, R., Gern, L., 1995. Strain variation of Lyme disease spirochetes isolated from *Ixodes ricinus* ticks and rodents collected in two endemic areas in Switzerland. J. Med. Entomol. 32, 433–438.
- Humair, P.F., Rais, O., Gern, L., 1999. Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. Parasitology 118, 33–42.
- Jouda, F., Perret, J.L., Gern, L., 2004. Density of questing *Ixodes ricinus* nymphs and adults infected by *Borrelia burgdorferi* sensu lato in Switzerland: spatio-temporal pattern at a regional scale. Vector-Borne Zoonot. Dis. 4, 23–32.
- Kurtenbach, K., Peacey, M., Rijpkema, S.G.T., Hoodless, A.N., Nuttall, P.A., Randolph, S.E., 1998. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. Appl. Environ. Microbiol. 64, 1169–1174.
- Lagal, V., Postic, D., Ruzic-Sabljić, E., Baranton, G., 2003. Genetic diversity among *Borrelia* strains determined by single-strand conformation polymorphism analysis of the *ospC* gene and its association with invasiveness. J. Clin. Microbiol. 41, 5059–5065.
- Lindsay, L.R., Barker, I.K., Surgeoner, G.A., McEwen, S.A., Campbell, G.D., 1997. Duration of *Borrelia burgdorferi* infectivity in white-footed mice for the tick vector *Ixodes scapularis* under laboratory and field conditions in Ontario. J. Wildl. Dis. 33, 766–775.
- Mackinnon, M.J., Gandon, S., Read, A.F., 2008. Virulence evolution in response to vaccination: The case of malaria. Vaccine 26, C42–C52.
- Mackinnon, M.J., Read, A.F., 1999. Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution 53, 689–703.
- Mbow, M.L., Christe, M., Rutti, B., Brossard, M., 1994. Absence of acquired resistance to nymphal *Ixodes ricinus* ticks in BALB/c mice developing cutaneous reactions. J. Parasitol. 80, 81–87.
- Morán Cadena, F., Rais, O., Jouda, F., Douet, V., Humair, P.-F., 2007a. Phenology of *Ixodes ricinus* and infection with *Borrelia burgdorferi* sensu lato along a North- and South-facing altitudinal gradient on Chaumont Mountain, Switzerland. J. Med. Entomol. 44, 683–693.
- Morán Cadena, F.M., Rais, O., Humair, P.F., Douet, V., Moret, J., Gern, L., 2007b. Identification of host bloodmeal source and *Borrelia burgdorferi* sensu lato in field-collected *Ixodes ricinus* ticks in Chaumont (Switzerland). J. Med. Entomol. 44, 1109–1117.
- Ohnishi, J., Piesman, J., de Silva, A.M., 2001. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. Proc. Natl. Acad. Sci. U. S. A. 98, 670–675.
- Pal, U., Yang, X.F., Chen, M., Bockenstedt, L.K., Anderson, J.F., Flavell, R.A., Norgard, M.V., Fikrig, E., 2004. *OspC* facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. J. Clin. Investig. 113, 220–230.
- Pérez, D., Kneubühler, Y., Rais, O., Jouda, F., Gern, L., 2011. *Borrelia afzelii* *ospC* genotype diversity in *Ixodes ricinus* questing ticks and ticks from rodents in two Lyme borreliosis endemic areas: Contribution of co-feeding ticks. Ticks Tick-Borne Dis. 2, 137–142.
- Piesman, J., Gern, L., 2004. Lyme borreliosis in Europe and North America. Parasitology 129, S191–S220.
- Piesman, J., Schneider, B.S., Zeidner, N.S., 2001. Use of quantitative PCR to measure density of *Borrelia burgdorferi* in the midgut and salivary glands of feeding tick vectors. J. Clin. Microbiol. 39, 4145–4148.
- Qiu, W.G., Dykhuizen, D.E., Acosta, M.S., Luft, B.J., 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the northeastern United States. Genetics 160, 833–849.
- Qiu, W.G., Schutzer, S.E., Bruno, J.F., Attie, O., Yun, X., Dunn, J.J., Fraser, C.M., Casjens, S.R., Luft, B.J., 2004. Genetic exchange and plasmid transfers in *Borrelia burgdorferi* sensu stricto revealed by three-way genome comparisons and multilocus sequence typing. Proc. Natl. Acad. Sci. U. S. A. 101, 14150–14155.
- Raberg, L., 2012. Infection intensity and infectivity of the tick-borne pathogen *Borrelia afzelii*. J. Evol. Biol. 25, 1448–1453.
- Radolf, J.D., Caimano, M.J., 2008. The long strange trip of *Borrelia burgdorferi* outer-surface protein C. Mol. Microbiol. 69, 1–4.
- Randolph, S.E., 1998. Ticks are not insects: consequences of contrasting vector biology for transmission potential. Parasitol. Today 14, 186–192.
- Randolph, S.E., 2011. Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm. Ticks Tick-Borne Dis. 2, 179–182.
- Randolph, S.E., Gern, L., Nuttall, P.A., 1996. Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parasitol. Today 12, 472–479.
- Randolph, S.E., Miklisova, D., Lysy, J., Rogers, D.J., Labuda, M., 1999. Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118, 177–186.
- Richter, D., Allgower, R., Matuschka, F.R., 2002. Co-feeding transmission and its contribution to the perpetuation of the Lyme disease spirochete *Borrelia afzelii*. Emerg. Infect. Dis. 8, 1421–1425.
- Richter, D., Postic, D., Sertour, N., Livey, I., Matuschka, F.R., Baranton, G., 2006. Delimitation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp nov. Int. J. Syst. Evol. Microbiol. 56, 873–881.
- Seinost, G., Dykhuizen, D.E., Dattwyler, R.J., Golde, W.T., Dunn, J.J., Wang, I.N., Wormser, G.P., Schriefer, M.E., Luft, B.J., 1999. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect. Immun. 67, 3518–3524.
- Sinsky, R.J., Piesman, J., 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J. Clin. Microbiol. 27, 1723–1727.
- Stearns, S.C., 1992. The evolution of life-histories. Oxford University Press, Oxford.
- Tilly, K., Krum, J.G., Bestor, A., Jewett, M.W., Grimm, D., Bueschel, D., Byram, R., Doward, D., VanRaden, M.J., Steward, P., Rosa, P., 2006. *Borrelia burgdorferi* *ospC* protein required exclusively in a crucial early stage of mammalian infection. Infect. Immun. 74, 3554–3564.
- Tilly, K., Rosa, P.A., Stewart, P.E., 2008. Biology of infection with *Borrelia burgdorferi*. Infect. Dis. Clin. North Am. 22, 217–234.
- Titus, R.C., Ribeiro, J.M.C., 1988. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance leishmania infectivity. Science 239, 1306–1308.
- Tsao, J., 2009. Reviewing molecular adaptations of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles. Vet. Res. (Paris) 40.
- Voordouw, M.J., 2015. Co-feeding transmission in Lyme disease pathogens. Parasitology 142, 290–302.
- Wang, G., Ojaimi, C., Iyer, R., Saksenberg, V., McClain, S.A., Wormser, G.P., Schwartz, I., 2001. Impact of genotypic variation of *Borrelia burgdorferi* sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. Infect. Immun. 69, 4303–4312.
- Wang, G.Q., Ojaimi, C., Wu, H.Y., Saksenberg, V., Iyer, R., Liveris, D., McClain, S.A., Wormser, G.P., Schwartz, I., 2002. Disease severity in a murine model of Lyme borreliosis is associated with the genotype of the infecting *Borrelia burgdorferi* sensu stricto strain. J. Infect. Dis. 186, 782–791.
- Wang, I.N., Dykhuizen, D.E., Qiu, W., Dunn, J.J., Bosler, E.M., Luft, B.J., 1999. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. Genetics 151, 15–30.
- Wikle, S.K., 1999. Tick modulation of host immunity: an important factor in pathogen transmission. Int. J. Parasitol. 29, 851–859.
- Wormser, G.P., Brisson, D., Liveris, D., Hanincova, K., Sandigursky, S., Nowakowski, J., Nadelman, R.B., Ludin, S., Schwartz, I., 2008. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. J. Infect. Dis. 198, 1358–1364.
- Zeidner, N.S., Schneider, B.S., Dolan, M.C., Piesman, J., 2001. An analysis of spirochete load, strain, and pathology in a model of tick-transmitted Lyme borreliosis. Vector Borne Zoonot. Dis. 1, 35–44.