Borrelia afzelii alters reproductive success in a rodent host

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Abstract

The impact of a pathogen on the fitness and behaviour of its natural host depends upon the host-parasite relationship in a given set of environmental conditions. Here, we experimentally investigated the effects of *Borrelia afzelii*, one of the etiological agents of Lyme disease in humans, on the fitness of its natural rodent host, the bank vole (*Myodes glareolus*), in semi-natural conditions with two contrasting host population densities. Our results show that *B. afzelii* can modify the reproductive success and spacing behaviour of its rodent host, whereas host survival was not affected. Infection impaired the breeding probability of large bank voles. Reproduction was hastened in infected females without alteration of the offspring size at birth. At low density, infected males produced fewer offspring, fertilised fewer females and had lower mobility than uninfected individuals. Meanwhile, the infection did not affect the proportion of offspring produced or the proportion of mating partner in female bank voles. Our study is the first to show that *B. afzelii* infection alters the reproductive success of the natural host. The effects observed can reflect the sickness behaviour due to the infection or they can be a consequence of a manipulation of the host behaviour by the bacteria.

1. Introduction

The impact of pathogens on the physiology, behaviour and fitness of their natural hosts is a key determinant for the co-evolution between the pathogen and the host [1–4]. Identifying the effect of a pathogen on all components of host fitness is also essential for predicting the population dynamics of a host-pathogen association and is fundamental for anticipating zoonotic outbreaks [5–8]. However, the study of the impact of parasites on their natural hosts often focuses on host survival [3,9–11], despite the recognition that host reproduction is an important component of host fitness [12–14]. Indeed, subtle effects of an endemic pathogen on the reproduction of its natural host can influence the population dynamics of the wild host [15,16] and ultimately the population dynamics of the pathogen [17].

Numerous studies have shown that pathogen virulence depends on ecological factors such as temperature and nutrition [18–21]. Another important ecological factor is host population density because it generates intra-specific competition for limited resources such as space, food and mating partners [22–24]. High host density is therefore expected to exacerbate pathogen virulence. Fluctuations in population density are typical in many small mammal species such as rodents [25]. However, experimental studies on density-dependent costs of infection in rodents are rare because it is often difficult to manipulate host density in an ecologically relevant way (but see [10,26,27]).

Spirochete bacteria belonging to the *Borrelia burgdorferi* sensu lato (s. l.) complex cause Lyme borreliosis in humans, which is the most common vector-borne disease in the northern hemisphere [28,29]. *Borrelia afzelii*, which is transmitted by *Ixodes* ticks and hosted by rodents, is the most common etiological agent of human Lyme borreliosis in Europe [28,30]. While Lyme

borreliosis causes serious morbidity in humans [31,32], there is currently no clear evidence that *Borrelia burgdorferi* s. l. reduces the fitness of the rodent or avian reservoir hosts [6,9,33–36]. However, most studies that investigate the virulence of *Borrelia burgdorferi* s. l. pathogens were correlational and have focused on host survival and, to date, the potential effects on host reproductive success have been ignored (but see [37,38] for physiological cost and effect on host behaviour, respectively).

We conducted a field experiment to test whether *B. afzelii* reduces the survival and reproductive success of its rodent host, the bank vole (*Myodes glareolus*). Rodent populations are often strongly influenced by density-dependent effects [25]. We, therefore, hypothesised that the detrimental effects of *B. afzelii* infection on the fitness of bank voles would be more pronounced at high population density. Here, we show that while *B. afzelii* did not affect the host survival, the infection impaired the reproduction of large bank voles, and unexpectedly, that male bank voles had lower reproductive performances at low population density.

2. Methods

(a) Ethical statement

The Finnish Animal Experiment Board approved the trapping and handling methods used in this study under the authorisations ESAVI/3834/04.10.03/2011, ESAVI/7256/04.10.07/2014 and ESAVI/3457/04.10.07/2015.

(b) Experimental design

The schedule of the experimental procedure is shown in Fig. S1, and all methods are detailed in the supplementary material. Male and female bank voles (*Myodes glareolus*) from the lab colony at the University of Jyväskylä were measured and assigned to either the *B. afzelii* infection group (injected with a local strain of *B. afzelii*) or the uninfected control group (injected with PBS). All infected and uninfected voles (total of 136 individuals, 68 females and 68 males) were released in 12 large outdoor vegetated enclosures (each 0.2 ha) that were assigned to "high" density (16 individuals per enclosure, 8 females and 8 males, half of each sex infected, 5 enclosures) and "low" density (8 individuals per enclosure, 4 females and 4 males, half of each sex infected, 7 enclosures) treatments. In the enclosures, the bank voles could move and reproduce freely for 18 days, which is the minimum gestation length in females. During this period, spacing behaviour was monitored using live trapping. At the end of this period, all trapped individuals were taken to the laboratory for measurements and monitoring of parturition. Male reproductive success was determined by paternity analyses conducted on the offspring born in the laboratory.

(c) Measurements

Before the enclosure period, individuals were weighed, and the head width was measured with a calliper ruler (Electronic Digital Caliper, Scala). These measurements were taken into account when experimental animals were assigned to treatments and enclosures. An ear tissue sample was taken for paternity analysis. A blood sample was taken for an ELISA targeting *B. burgdorferi* s. l.-specific IgG antibodies [39] (supplementary material).

After the enclosure period, the body measurements and blood sampling were carried out as described above. Males were processed shortly after the trapping day, gravid females were processed after parturition, and females that were not gravid were processed at the end of the experiment. Pups were measured (body mass and head width) within 24 hours of parturition. All measurements were performed blind regarding the infection treatment and density treatment.

(d) Statistical analysis

All statistical analyses were carried out using the statistical software R version 3.1.1. Survival of bank voles in the enclosures and individual breeding probability are binary variables. For survival, individuals were assigned 0 or 1 depending on whether they were trapped at the end of the experiment or not. For the assessment of the breeding probability, individuals were assigned 0 or 1 depending on whether their number of produced offspring was zero or at least one. Moreover, two response variables of reproductive success were calculated: (1) 'relative number of offspring' is the proportion of offspring produced in an enclosure by a given individual, (2) 'relative number of partners' is the proportion of partners with which a given individual produced offspring. Eventually, space trapping data allowed to calculate two different home range variables: home range perimeter (m) and home range surface (m^2) (Table S1).

In the statistical analyses, the injection (*Borrelia afzelii* vs. PBS) was used to define "infection" treatment (infected vs. uninfected), and the population density in the enclosure defined the "density" treatment ("low" vs. "high"). The explanatory variables of the full models always included the two experimentally manipulated factors, *i.e.* the infection treatment and the population density in the enclosure, sex, body mass before injection (BM) and relevant two- and

three-way interactions. Enclosure ID was included as a random effect in all models. Three-way interactions involving vole sex were expected in models assessing bank vole reproductive success because the drivers of reproductive success differ between male and female bank voles [40–43]. When these three-way interactions were significant in the full model (see Table S1), separate analyses were conducted for males and females to ease the interpretation of the interactions. Otherwise, reductions of the full models were carried out starting from the non-significant interactions (see supplementary material).

For gravid females, the parturition delay was calculated as the difference in the number of days between the date the first litter was observed and the parturition date for the other pregnant females. This variable was modelled as a function of infection, density, BM, and the interaction infection × density. Moreover, offspring body mass at birth and head width at birth were modelled as a function of the infection status of the mother and father, density and all their two- and three-way interactions. Offspring sex and litter size were included as covariates. Enclosure ID, mother ID and father ID were included as random effects.

To analyse the data, we used generalized linear mixed models (GLMMs) with an error distribution that was either normal (home range perimeter, home range surface, body mass and head width of offspring), binomial (survival, breeding probability and variables describing reproductive success: relative number of offspring and relative number of partners) or negative binomial (female parturition delay).

3. Results

Out of the 68 female and 68 male bank voles released into the enclosures at the beginning of the experiment, 48 females and 30 males (one of which was found dead in the trap) were recovered, and the remaining 58 individuals were considered as dead. Of these 58 individuals, 56 were never observed during the 14 trapping occasions and 2 were not observed during the six last trapping occasions. As we did not observe any introduction of unmarked wild bank voles in the enclosures, and all trapped animals were found in their original enclosure, we assume that missing animals died, rather than escaped. Of the 78 captured individuals, 39 were from the *B. afzelii* infection group (24 females, 15 males), and 39 were from the control group (24 females, 15 males, including the individual found dead in the trap). There was no effect of *B. afzelii* infection or population density on the survival of bank voles (GLMM: $p > 0.35$, Table S4), but females survived better than male bank voles (GLMM: $p < 0.01$, Table S4).

(a) *B. afzelii* infection reduces the breeding probability of large bank voles

Based on the paternity test, 39 of 68 males reproduced during the study (18 of the 30 males that were trapped and 21 of the 38 males that were not trapped). For the analysis of reproductive success, all males were included, regardless of whether they were trapped or not at the end of the study. Out of the 48 captured females, 45 gave birth in the laboratory. We found that the effect of *B. afzelii* infection on bank vole breeding probability was dependent on body size: among small individuals, there was no difference in the breeding probability between infected and uninfected animals. However, uninfected individuals had significantly higher breeding probability than *B. afzelii*-infected individuals among large bank voles (GLMM: body mass × infection, $p = 0.05$,

Table 1 and Fig. 1; Table S4 and Fig S4).

(b) *B. afzelii* infection reduces male reproductive success at low density

Reproductive success was further explored as the analysis of the relative number of produced offspring and the relative number of partners. The three-way interaction infection × density × sex was significant for the relative number of offspring (GLMM: $p = 0.02$, Table S1) and the relative number of partners (GLMM: $p = 0.03$, Table S1), providing evidence that infection and breeding density affected these components of reproductive success differently in males and females. In male bank voles, the relative number of offspring and partners were associated with *B. afzelii* infection status, but the effect differed between the population density treatments (Table 1, Fig. 2, Table S2). In low density, uninfected control males sired a higher relative number of offspring (0.42) and fertilised a higher relative number of females (0.43) than *B. afzelii*-infected males (0.05 offspring sired and 0.10 female fertilised). Conversely, in high density there was no effect of the infection treatment: the relative number of offspring sired by uninfected and infected males were 0.13 and 0.11, and the relative number of females fertilised by uninfected and infected males were 0.18 and 0.19, respectively (GLMM: $p = 0.004$ and $p = 0.02$, Table 1, Fig. 2). For female bank voles, the relative number of offspring and partners were not affected by the infection (the proportion of offspring produced by uninfected and infected females was 0.27 and 0.26, respectively; GLMM: $p = 0.79$, Table S3). As expected, population density influenced the relative number of offspring produced by a female bank vole (relative number of offspring produced by females from low and high-density enclosures was 0.41 and 0.18, respectively; GLMM: estimate on the logit scale (SE): density = 0.85 (0.25), $p < 0.001$, Table S3).

(c) *B. afzelii* infection reduces male home range at low density

We found evidence that male and female bank voles differ in their spacing behaviour as the three-way interaction infection × density × sex was significant for home range surface and home range perimeter (GLMM: $p < 0.01$, $p = 0.03$, respectively, Table S1). For the uninfected male bank voles, the home range surface was significantly larger in the low-density enclosures (808 m^2) compared to the high-density enclosures (378 m^2) (LMM: $p = 0.003$, Table 1, Fig. 3). In contrast, the home range surface of the *B. afzelii*-infected male bank voles was not significantly different between the low density (360 m^2) and high density (524 m^2) enclosures (Table 1, Fig. 3). Female home range surface and perimeter were not affected by the infection or the density treatments (Table S3).

(d) Infection caused early reproduction in female bank voles

Of the 48 females captured from the enclosures, 45 were gravid and produced a total of 226 pups, with a mean number of 5 pups per female (range: 1–7). *B. afzelii*-infected females reproduced on average 3 days earlier than uninfected control females (GLMM: $p = 0.003$, Fig. 4, Table 1) and this effect was independent of the population density (GLMM: $p = 0.30$, Table 1). The size of the offspring at birth was not affected by the infection treatment of the mother or father or population density (LMM for all variables: $p > 0.05$, Table S6).

4. Discussion

We examined the hypothesis that *B. afzelii* infection reduces the reproductive success of the rodent host and we tested the density-dependence of this effect. We found that *B. afzelii* infection had density-dependent and statistically differing effects on the relative numbers of partners and offspring of male and female bank voles. In males, infected individuals kept at low population density sired a lower proportion of offspring, fertilised a lower proportion of females and displayed smaller home range surface than uninfected males (Fig. 2, 3). In females, by contrast, *B. afzelii* infection did not affect the relative offspring number, relative number of partners and home range surface, but infected individuals gave birth *ca.* 3 days earlier than uninfected individuals. The offspring size (head width and body mass) was not affected by the mother's infection status (Fig. 4, Table S6). Finally, in both sexes, infection reduced the breeding probability of large individuals but did not affect their survival (Fig. 1, Table S4, Table S5).

Previous studies found no evidence that infection with *Borrelia burgdorferi* s. l. reduces the fitness of natural hosts; however, most of them were correlational or focused on another genospecies than *B. afzelii*. For instance, capture-mark-recapture (CMR) studies on wild populations of the white-footed mouse (*Peromyscus leucopus*) or the black-legged kittiwake found no effect of infection with *B. burgdorferi* s. l. on the survival of these hosts [9,34,35]. Similarly, we found that infection with *B. afzelii* did not impair survival of the bank vole. Another study on white-footed mice found no effect of *B. burgdorferi* s. s. on the wheel running behaviour over the six weeks following experimental infection [6]. In our study, by contrast, the effect of infection on home range size may result from altered running behaviour. A recent study reports a trend in increased foraging behaviour in white-footed mice treated with an anti-*B.*

231 *burgdorferi* vaccine compared with sham-treated individuals, suggesting similarly to our finding,
232 a wider ranging behaviour in individuals with low or with no infection burden [38]. To our
233 knowledge, our study is the first to address the effect of *B. afzelii* infection on host reproduction
234 experimentally under field conditions. Studying the effects of infections on host reproduction is
235 challenging in wild rodent populations, and reproduction is often a latent variable inferred from
236 observed variables. Our experimental setting allows controlling for several sources of variation
237 and confounding factors (*e.g.* age of the host), and we were able to estimate the reproductive
238 success reliably.

239 The experimental infection was performed by peritoneal injection of the bacteria rather
240 than the natural infection route, which involves *Ixodes* ticks. The infection dose and route were
241 based on the literature [33,36,44–46]. The intraperitoneal route was chosen as it has been shown
242 to give more widely disseminated infection than the subcutaneous route [47]. The use of
243 injection instead of the natural transmission route can be debatable, *e.g.* due to the lack of tick
244 salivary compounds that enhance the infectivity of *Borrelia burgdorferi* s. l. [48,49]. Molecules
245 present in tick saliva promote the infection by manipulating or depressing the immune system
246 (*e.g.* Salps) [50]. The injection of *B. burgdorferi* s. l. with tick salivary gland extract led to higher
247 infection success with higher bacterial dissemination, so-called saliva-assisted transmission
248 [50,51]. The lack of these molecules could lead to misestimation of the effects of the infection on
249 the host. However, the injection allows the experimenter to control for the bacterial dose, and it
250 eliminates the variation linked to the tick vectorial capacity [52], hence ensuring a controlled
251 exposure of the study animals to the bacteria. We acknowledge that needle inoculation mimics
252 only grossly the infection via tick bite. However, we can expect any observed effect to be caused

253 by the *B. afzelii* infection given our controlled experimental conditions.

254 The demonstration of fitness-related costs caused by *B. burgdorferi* s. l. infection is
255 important for understanding the evolution of resistance in natural hosts. Recent field studies on
256 the bank vole suggest that polymorphism at the Toll-like receptor 2 (TLR2) gene, a pathogen
257 recognition receptor of the innate immune system, was associated with variation in susceptibility
258 to *B. afzelii* [53,54]. The prevalence of *B. afzelii* infection in bank voles that were homozygous
259 for the C2 resistance allele was half that of the bank voles that were homozygous for the C1
260 susceptibility allele [53]. A study of the TLR2 polymorphism in bank vole populations across
261 Europe found that the resistance allele against *B. afzelii* (C2) was more common in countries
262 with a high incidence of human Lyme disease [55]. This result led Tschirren (2015) to suggest
263 that *B. afzelii* was driving the evolution of the resistance allele at the TLR2 gene in European
264 bank vole populations. However, without clear evidence of reduced fitness in infected rodents,
265 the mechanism of selection was unclear. Our demonstration that infection with *B. afzelii* reduces
266 male reproductive success supports the hypothesis that this pathogen could be driving selection
267 on the TLR2 gene in bank vole populations.

268 The effect of the infection on the relative number of offspring sired and the relative
269 number of females fertilised by a given male bank vole was density-dependent. In the low-
270 density populations, uninfected control males fertilised more females and fathered more
271 offspring compared to the infected males and males kept in high population density (Fig. 2). This
272 result was counter-intuitive, as we predicted that the negative effects of high population density,
273 such as reduced per capita food availability, more aggressive interactions, and potentially higher
274 stress levels, would exacerbate the cost of *B. afzelii* infection [10,42,56–58]. Three hypotheses

275 can explain this result. First, several studies have shown that the strength of male-male
276 competition can vary with population density in a non-linear fashion [See, for instance, 59–61].
277 For example, males can modify their reproductive strategy in high population density leading to
278 lower rates of aggression and lower reproductive success [59,62]. Second, as estimates of the
279 relative number of partners and the relative number of offspring were based on paternity tests,
280 cryptic female choice (*i.e.* a female choice that occurs in the reproductive tract of the female,
281 leading to fertilisation bias in favour of specific males [63,64]) might have occurred. Thus, a
282 density-dependent female cryptic choice favouring healthy males in low-density populations
283 cannot be excluded. Finally, a spurious effect linked to the length of our experiment, which
284 covers only one reproductive episode, cannot be ruled out [65].

285 In the low-density populations, uninfected control males had larger home range sizes than
286 infected males whereas, in the high-density enclosures, there was no significant difference in the
287 home range size between uninfected and infected male bank voles (Fig. 3). One possible
288 explanation for this density-dependent home range reduction is that at high density, males may
289 reduce their exploratory behaviour to avoid encountering other males and having to engage in
290 aggressive male-male interactions. Moreover, at high density, with eight females available in the
291 enclosure, the chance for a male to encounter a receptive female might be higher than in the low-
292 density enclosure where only four females are available. Indeed, female bank voles are territorial
293 and hyperdispersed [41,66]. Consequently, at low density, male bank voles may need to explore
294 a larger home range to search for receptive females than at high density. As expected, the
295 uninfected males had a larger home range in low population density, whereas the infected males
296 presumably allocated resources to their immune response instead of explorative behaviour. In

297 contrast, female bank voles had a smaller home range size than males, that was not affected by
298 population density, reflecting the territorial behaviour of females especially, during late gestation
299 when the space trapping took place [41,66,67].

300 We found that the cost of infection was more important in large bank voles, which are the
301 most frequently infested with ticks and *B. burgdorferi* s. l. in nature [9,68,69]. Large infected
302 individuals showed reduced reproductive success compared to large healthy individuals. Food
303 resource is generally known to constrain reproduction and food addition has been shown to
304 enhance reproductive success in similar outdoor enclosure setups [42,70,71]. These food
305 constraints might have a more negative effect on the large individuals, which have greater
306 energetic needs [72]. Infected large voles showed altered breeding probability regardless of the
307 population density.

308 Infected females plastically modified their life history and reproduced *ca.* 3 days earlier
309 than uninfected females without alteration of the size of the offspring at birth, *i.e.* without signs
310 of premature birth (Fig. 4, Table 1, Table S3). In nature, reproducing females give birth to 1 or 2
311 litters per reproductive season [73], and most individuals live only one season. The biological
312 importance of giving birth three days earlier is not clear, as concerns population dynamics. At
313 the individual level, early reproduction can be a compensatory strategy if parasites reduce the
314 reproductive success of the adult host later in life via morbidity, mortality or castration [74–76].
315 According to the terminal investment theory, individuals maximise their fitness by allocating
316 resources to immediate reproduction when the prospects for future reproduction are reduced, for
317 example by chronic infection [27,77–79]. It remains to be estimated whether *B. afzelii* impairs
318 reproduction of female bank vole during the late stage of infection.

In summary, our study shows, for the first time, that the zoonotic pathogen *B. afzelii* can influence the reproductive success of its rodent host. The effect of the infection on the relative number of offspring and partners differed between male and female bank voles. Although large body size favoured reproduction in uninfected individuals, this size benefit disappeared if the individual was infected with *B. afzelii*. In males, infected individuals kept at low population density displayed smaller home range surface than uninfected males. Lower mobility can be a consequence of sickness behaviour due to the infection. On the other hand, predation risk by small carnivores generally increases with vole mobility [80]. By reducing home range size, infection with *B. afzelii* could lower the predation risk of male bank voles by small carnivores, enhancing at the same time, its own fitness [81]. The hypothesis of manipulation of the rodent host by *B. afzelii* is yet to be explored.

Supplementary information is available for this paper.

Data accessibility statement: The dataset analysed during the current study is available in the JYX repository, http://urn.fi/URN:NBN:fi:jyu-201806133148 [82].

Competing interests: The authors declare no competing financial interests.

Authors' contributions: C.C., E.K., T.M., E.R.K. conceived the study. The fieldwork and laboratory work were carried out by all authors, with the helpers listed in the Acknowledgements. C.C., E.K., T.M., E.R.K., M.V. analysed the results, with input from the

other authors. All authors contributed to the interpretation and critical revision. C.C. led the writing of the paper. All authors gave final approval for publication.

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Figure captions

Figure 1. The estimated probability of reproduction for a bank vole (± 95% CI) depends on their *B. afzelii* infection treatment (uninfected individuals in black, N = 68, infected individuals in grey, N = 68) and their body size (measured as the body mass before injection). In small bank voles, there is no effect of *B. afzelii* infection on breeding probability. In large bank voles, by contrast, uninfected individuals have higher breeding probability than infected individuals. The observed values are shown with open circles.

Figure 2. The estimated reproductive success of male bank voles depends on the interaction between *B. afzelii* infection (uninfected individuals in black, N = 34, infected individuals in grey, N = 34) and population density. (A) Predicted proportion of females successfully fertilised by a male bank vole (± 95% CI) as a function of infection and density. (B) Predicted proportion of offspring sired by a male bank vole (± 95% CI) as a function of infection and density (Table S2). The observed values are shown with open circles.

Figure 3. The estimated home range (in m^2) of male bank voles in the enclosures (± 95% CI) depends on the interaction between *B. afzelii* infection (uninfected individuals in black, N = 13, infected individuals in grey, N = 14) and population density. At low population density, uninfected males have much larger home ranges than *B. afzelii*-infected males. At high population density, infection with *B. afzelii* does not affect the home range of male bank voles (Table S2). The observed values are shown with open circles.

Figure 4. Estimated parturition delay in female bank voles (± 95% CI) depends on *B. afzelii* infection (uninfected individuals in black, N = 23, infected individuals in grey, N = 22) and population density (Table 1 and S4). The observed values are shown with open circles.

Table caption

Table 1: Selected final models for reproductive success and spacing behaviour in bank voles. BM: centred value of body mass before injection; HW: centred value of head width before injection, low: low population density, inf: infected bank voles; $\sigma 2$ is the variance attributable to random effect, SD is the standard deviation, SE is the standard error. **Significant effects are shown in bold.**