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**Title:** *Borrelia afzelii* alters reproductive success in a rodent host

**Year:** 2018

**Version:** Accepted version (Final draft)

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**Please cite the original version:**

Cayol, C., Giermek, A., Gomez-Chamorro, A., Hytönen, J., Kallio, E., Mappes, T., Salo, J., Voordouw, M. J., & Koskela, E. (2018). *Borrelia afzelii* alters reproductive success in a rodent host. *Proceedings of the Royal Society B : Biological Sciences*, 285(1884), Article 20181056. <https://doi.org/10.1098/rspb.2018.1056>

1 ***Borrelia afzelii* alters reproductive success in a rodent host**

2  
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20  
21 **Keywords:** *Borrelia afzelii*; fitness; host-pathogen interaction; *Myodes glareolus*; zoonosis;  
22 natural host

23        **Abstract**

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

## 38 **1. Introduction**

39 The impact of pathogens on the physiology, behaviour and fitness of their natural hosts is a key  
40 determinant for the co-evolution between the pathogen and the host [1–4]. Identifying the effect  
41 of a pathogen on all components of host fitness is also essential for predicting the population  
42 dynamics of a host-pathogen association and is fundamental for anticipating zoonotic outbreaks  
43 [5–8]. However, the study of the impact of parasites on their natural hosts often focuses on host  
44 survival [3,9–11], despite the recognition that host reproduction is an important component of  
45 host fitness [12–14]. Indeed, subtle effects of an endemic pathogen on the reproduction of its  
46 natural host can influence the population dynamics of the wild host [15,16] and ultimately the  
47 population dynamics of the pathogen [17].

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

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

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

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

73

## 74 **2. Methods**

### 75 **(a) Ethical statement**

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

79

80 **(b) Experimental design**

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

94

95 **(c) Measurements**

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

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

106

#### 107 **(d) Statistical analysis**

108 All statistical analyses were carried out using the statistical software R version 3.1.1. Survival of  
109 bank voles in the enclosures and individual breeding probability are binary variables. For  
110 survival, individuals were assigned 0 or 1 depending on whether they were trapped at the end of  
111 the experiment or not. For the assessment of the breeding probability, individuals were assigned  
112 0 or 1 depending on whether their number of produced offspring was zero or at least one.

113 Moreover, two response variables of reproductive success were calculated: (1) ‘relative number  
114 of offspring’ is the proportion of offspring produced in an enclosure by a given individual, (2)  
115 ‘relative number of partners’ is the proportion of partners with which a given individual  
116 produced offspring. Eventually, space trapping data allowed to calculate two different home  
117 range variables: home range perimeter (m) and home range surface (m<sup>2</sup>) (Table S1).

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

123 three-way interactions. Enclosure ID was included as a random effect in all models. Three-way  
124 interactions involving vole sex were expected in models assessing bank vole reproductive  
125 success because the drivers of reproductive success differ between male and female bank voles  
126 [40–43]. When these three-way interactions were significant in the full model (see Table S1),  
127 separate analyses were conducted for males and females to ease the interpretation of the  
128 interactions. Otherwise, reductions of the full models were carried out starting from the non-  
129 significant interactions (see supplementary material).

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

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

142

143



144       **3. Results**

145    Out of the 68 female and 68 male bank voles released into the enclosures at the beginning of the  
146    experiment, 48 females and 30 males (one of which was found dead in the trap) were recovered,  
147    and the remaining 58 individuals were considered as dead. Of these 58 individuals, 56 were  
148    never observed during the 14 trapping occasions and 2 were not observed during the six last  
149    trapping occasions. As we did not observe any introduction of unmarked wild bank voles in the  
150    enclosures, and all trapped animals were found in their original enclosure, we assume that  
151    missing animals died, rather than escaped. Of the 78 captured individuals, 39 were from the *B.*  
152    *afzelii* infection group (24 females, 15 males), and 39 were from the control group (24 females,  
153    15 males, including the individual found dead in the trap). There was no effect of *B. afzelii*  
154    infection or population density on the survival of bank voles (GLMM:  $p > 0.35$ , Table S4), but  
155    females survived better than male bank voles (GLMM:  $p < 0.01$ , Table S4).

156

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

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

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

167

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

169 Reproductive success was further explored as the analysis of the relative number of produced  
170 offspring and the relative number of partners. The three-way interaction infection  $\times$  density  $\times$  sex  
171 was significant for the relative number of offspring (GLMM:  $p = 0.02$ , Table S1) and the relative  
172 number of partners (GLMM:  $p = 0.03$ , Table S1), providing evidence that infection and breeding  
173 density affected these components of reproductive success differently in males and females. In  
174 male bank voles, the relative number of offspring and partners were associated with *B. afzelii*  
175 infection status, but the effect differed between the population density treatments (Table 1, Fig.  
176 2, Table S2). In low density, uninfected control males sired a higher relative number of offspring  
177 (0.42) and fertilised a higher relative number of females (0.43) than *B. afzelii*-infected males  
178 (0.05 offspring sired and 0.10 female fertilised). Conversely, in high density there was no effect  
179 of the infection treatment: the relative number of offspring sired by uninfected and infected  
180 males were 0.13 and 0.11, and the relative number of females fertilised by uninfected and  
181 infected males were 0.18 and 0.19, respectively (GLMM:  $p = 0.004$  and  $p = 0.02$ , Table 1, Fig.  
182 2). For female bank voles, the relative number of offspring and partners were not affected by the  
183 infection (the proportion of offspring produced by uninfected and infected females was 0.27 and  
184 0.26, respectively; GLMM:  $p = 0.79$ , Table S3). As expected, population density influenced the  
185 relative number of offspring produced by a female bank vole (relative number of offspring  
186 produced by females from low and high-density enclosures was 0.41 and 0.18, respectively;  
187 GLMM: estimate on the logit scale (SE): density = 0.85 (0.25),  $p < 0.001$ , Table S3).

188

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

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

199

200 **(d) Infection caused early reproduction in female bank voles**

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

207

208

209 **4. Discussion**

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

221 Previous studies found no evidence that infection with *Borrelia burgdorferi* s. l. reduces  
222 the fitness of natural hosts; however, most of them were correlational or focused on another  
223 genospecies than *B. afzelii*. For instance, capture-mark-recapture (CMR) studies on wild  
224 populations of the white-footed mouse (*Peromyscus leucopus*) or the black-legged kittiwake  
225 found no effect of infection with *B. burgdorferi* s. l. on the survival of these hosts [9,34,35].  
226 Similarly, we found that infection with *B. afzelii* did not impair survival of the bank vole.  
227 Another study on white-footed mice found no effect of *B. burgdorferi* s. s. on the wheel running  
228 behaviour over the six weeks following experimental infection [6]. In our study, by contrast, the  
229 effect of infection on home range size may result from altered running behaviour. A recent study  
230 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.



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

330

331 **Supplementary information is available for this paper.**

332

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

335

336 **Competing interests:** The authors declare no competing financial interests.

337

338 **Authors' contributions:** C.C., E.K., T.M., E.R.K. conceived the study. The fieldwork and  
339 laboratory work were carried out by all authors, with the helpers listed in the

340 Acknowledgements. C.C., E.K., T.M., E.R.K., M.V. analysed the results, with input from the

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

343

344 **Acknowledgements:** We thank Anja Siukkola, Susanne Varjola and Joannes Van Cann and the  
345 Konnevesi research station for their assistance in the field. We thank Sami Kyröläinen, Elina  
346 Virtanen, Annukka Pietikäinen, Julia Honkasalo, Anouk Sarr and Olivier Rais for their help in  
347 the lab. Swanne Gordon gave constructive feedback at an early stage of this manuscript. We  
348 thank Maarten Voordouw's and Johanna Mappes's lab meetings for their comments. We thank  
349 two anonymous referees for valuable comments on the manuscript.

350

351 **Fundings:** This project was supported by the Kone Foundation, the University of Jyväskylä and  
352 the Academy of Finland (Eva R. Kallio 250524, 310104; Esa Koskela 257340 and Tapio Mappes  
353 132190, 268670).

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- 570

571 **Figure captions**

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

578

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

585

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

592

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

596

597 **Table caption**

598 Table 1: Selected final models for reproductive success and spacing behaviour in bank voles.

599 BM: centred value of body mass before injection; HW: centred value of head width before  
600 injection, low: low population density, inf: infected bank voles;  $\sigma^2$  is the variance attributable to  
601 random effect, SD is the standard deviation, SE is the standard error. **Significant effects are**  
602 **shown in bold.**