Supplementary material

Borrelia afzelii alters reproductive success in a rodent host

Published in Proceedings of the Royal Society B.

Claire Cayol[1]*, Anna Giermek[2], Andrea Gomez-Chamorro[3], Jukka Hytönen[4], Eva R. Kallio[1,5], Tapio Mappes[1], Jemiina Salo[4], Maarten Jeroen Voordouw[3], Esa Koskela[1]

[1]Department of Biological and Environmental Science. P.O. Box 35, University of Jyväskylä, FI-40014, Jyväskylä, Finland. [2]Institute of Environmental Sciences. Gronostajowa 7, 30-387, Jagiellonian University, Cracow, Poland. [3]Institut de Biologie, Laboratoire d'Ecologie et Evolution des Parasites. Rue Emile-Argand 11, Université de Neuchâtel, CH-2000 Neuchâtel, Switzerland. [4]Institute of Biomedicine. Kiinamyllynkatu 13, University of Turku, FI-20520 Turku, Finland. [5]Department of Ecology and Genetics, P.O. Box 3000, FI-90014 University of Oulu, Oulu, Finland. *Correspondence and requests for materials should be addressed to C.C. (email: claire.c.cayol@jyu.fi, claire.c.cayol@gmail.com)

I. Supplementary text: Details of the materials and methods

A. Isolation of the local strain of *Borrelia afzelii*

The local strain of *B. afzelii* was isolated from wild bank voles (*Myodes glareolus*) that had been captured in urban forests around Jyväskylä, Finland.

1. Vole sampling

Wild adult male bank voles (n = 24) were live-trapped in urban forests around Jyväskylä, Finland, between September and October 2014. On the day of capture, an ear tissue biopsy (1.3 mg) was taken from the inside part of the ear with a biopsy punch and stored in ethanol at -20 °C (see [1]). Six bank voles from a Borrelia-free laboratory colony were similarly sampled for skin. These individuals served as negative controls for the DNA extraction and subsequent PCR.

2. DNA extraction, PCR and sequencing

DNA was extracted from the ear biopsy samples using the Genejet Genomic DNA purification kit (Thermo Fischer Scientific, Waltham, United States) following the manufacturer's instructions (Thermoscientific #K0721, #K0722). The amounts of some reagents were reduced due to the small sample size. Specifically, the volumes of proteinase K, RNase A, lysis solution, ethanol, and elution buffer were reduced to 10 µl, 10 µl, 100 µl, 200 µl, and 100 µl, respectively. The DNA extractions were stored at -20 °C until further processing.

Nested PCR targeting the *flagellin* gene (encoding the flagellar protein) was performed on the extracted DNA following the method of Wodecka *et al.* [2] slightly modified as follows. The total volume of the first PCR reaction was 10 µl and contained 0.025 U/µl of DreamTaq DNA polymerase (Thermo Scientific), 0.2 µM of each primer, 0.25 µg/µl of BSA, 200 µM dNTP, 1X of DreamTaq reaction buffer (Thermo Scientific), 1 µl of template, and topped up with sterile water. For the 24 field-captured males, 7 of the 24 ear tissue biopsies were positive for the

flagellin gene in the PCR. The expected 604 bp PCR product was sequenced (Prism 3130xl, Applied Biosystems, Foster City, United States) and the sequences were BLASTed against sequences of *Borrelia burgdorferi* sensu lato in GenBank (GenBank/EMBL/DDBJ sequences accession numbers for *B. burgdorferi* sensu stricto G112-04, *B. afzelii* D131-07, *B. garinii* G23-07, *B. valaisiana* G43-0507, and *B. miyamotoi* D110-07 were FJ518808, FJ518805, FJ518806, FJ518807 and FJ518804, respectively). The seven *flagellin* gene sequences obtained showed 98% or more similarity with *B. afzelii*.

3. Bacterial culture and strain level identification

Three of the *Borrelia*-positive bank voles were sacrificed and the ear skin, bladder, heart, spleen and kidney were collected aseptically and placed in Barbour-Stoenner-Kelly II (BSK II) medium enriched with phosphomycin (50 mg/ml, Sigma-Aldrich, St. Louis, United States), rifampicin (100 mg/ml, Sigma-Aldrich) and amphotericin B (5 mg/ml, Sigma-Aldrich). Sealed tubes were incubated at +37 °C in an incubation chamber and checked once per week under a dark-field microscope. Spirochete-shaped bacteria were present in two of the cultures, and they were stored in 20% (v/v) glycerol at -80 °C after 1 to 8 passages. The isolates were genotyped with respect to the highly polymorphic *ospC* gene [3–5]. The *ospC* gene sequences can be classified into *ospC* major groups (oMGs) following the nomenclature developed by Bunikis *et al.* [6]. Isolates of *B. afzelii* often contain strains carrying different oMGs; therefore we used 454-sequencing to test the purity of the two isolates in the present study [4]. This approach obtained 137 and 167 *ospC* gene sequences that were 99.3% (136/137) and 100.0% (167/167) pure for oMGs A3 and A9, respectively. The two isolates were therefore named Fin-Jyv-A3 and Fin-Jyv-A9, respectively. In the present study, only isolate Fin-Jyv-A3 was used in experimental infections. This isolate has been registered in the *Borrelia* MLST database under ID number 1961 and has multi-locus

sequence type (MLST) ST676.

Three weeks before the start of the experiment, the cultures were reactivated, and the bacterial concentration was assessed using a Neubauer chamber and a phase contrast microscope. Concentration was adjusted to 10^6 spirochetes per ml in phosphate-buffered saline (PBS) less than one hour before injection.

B. Details of the experimental infection

Voles in the infection group were injected intraperitoneally with ca. 10^5 bacteria of a local strain of *B. afzelii* suspended in 0.1 ml of phosphate buffered saline (PBS). The dose and infection route were based on the literature [7–11]. Voles belonging to the uninfected control group were injected intraperitoneally with 0.1 ml of PBS. Infection success of *B. afzelii* was confirmed by seroconversion; the mean absorbance in the ELISA was significantly different between the infected and the uninfected individuals (ANOVA: $F_{df1, df2=3.02}$, $p < 0.001$, Fig. S2), but not between sex (ANOVA: $F_{df1, df2 = 0.04}$, $p = 0.20$). The percentage of trapped individuals exposed to *Borrelia afzelii* that seroconverted was 87% (34/39).

C. Details of the animals used in the experiment

Between the end of January and March 2015, bank voles from a lab colony (descendants of wild-caught individuals, mainly first and second generation) were bred in the animal facility at the University of Jyväskylä. Bank voles were kept in standard mouse cages (43 cm*26 cm*15 cm) with wood shavings and under 16 h light:8 h dark photoperiod and temperature of ~19 °C. Animals had *ad libitum* access to food and water. Offspring were separated from their mothers

after weaning (21 days) and were kept in sibling groups according to their sex. Each individual was identified with a microchip inserted subcutaneously. At 20 days before the release in outdoor enclosures, all individuals were measured, sampled (see below), and housed individually.

D. Details concerning the enclosures

Enclosures were located in Konnevesi (Central Finland, 62.627907N, 26.291793E). Each enclosure had a surface area of 50 m*40 m and was surrounded by a metal fence, which prevented immigration and emigration of voles but did not prevent potential predation by raptors. For details concerning the enclosures and the vegetation, see [12]. The bank vole density in high- and low-density enclosures was 80 and 40 individuals per hectare respectively. In peak years, the bank vole population density might approach 40 individuals per hectare in the study area, but peak population density of 100 voles per hectare was recorded in Central Europe [13–15]. Therefore, our “low density” (40 individuals per hectare) corresponds to a high population density population in nature whereas our “high density” (80 individuals per hectare) approach the largest density observed in nature.

The mean body mass and mean head width were similar between the infection group and the control group for both males and females (male body mass: $t = 0.02$, $p = 0.98$; male head width: $t = -1.05$, $p = 0.30$; female body mass: $t = -0.05$, $p = 0.96$; female head width: $t = 0.20$, $p = 0.84$, Fig. S3). In addition, enclosures were similar with regards to individual body mass (males: $F = 1.4$, $p = 0.18$; females: $F = 1.08$, $p = 0.40$) and number of siblings in the litter of each bank vole (Kruskal-Wallis test = 9.71, $p = 0.94$). Moreover, individuals from the same litter were assigned into different enclosures to randomize any maternal effect and avoid kin recognition

and breeding among siblings. Thus, any observed differences in reproductive performance are likely to result from differences in infection or density treatment or both.

E. Space trapping and home range size

Space trapping allowed the computation of the home range perimeter (metres) and home range surface (m^2) for each individual vole. Each enclosure contained a network of 20 Ugglan Special live traps (Grahnab AB, Gnosjö, Sweden). The trap density was 100 traps/ha, and traps were spaced 10 m from each other and 5 m from the fences of the enclosures. During the trapping (13-18 days after the release of the voles into the enclosures), the traps were baited with sunflower seeds and potatoes, and they were checked 2 to 3 times per day for a total of 14 trap checks. Trapping locations were recorded for each individual before the immediate release of the animal. At the end of the trapping period, all the surviving individuals were brought into the animal facility for general measurements and monitoring of parturition.

To estimate the home range of the bank voles with the statistical package adehabitatHR, a matrix of detectors was created with 20 capture points spaced 10 m apart, as observed in the enclosures. Another matrix contained one row per animal and one column per occasion (capture session). Individuals trapped during a specific capture session were assigned a value of 1, whereas individuals not trapped were assigned a value of 0. Home range area and perimeter were computed for individuals caught 5 times or more using 95% of the relocations, with the cluster approach from the package adehabitatHR [16], which relies on the calculation of single-linkage cluster analysis as described by Kenward *et al* [17].

F. Additional analysis: Dual X-ray measurement

Body composition of each animal (*i.e.* body fat content and lean mass) was assessed by dual X-ray using a Lunar PIXImus Densitometer (GE Medical Systems), after the enclosure period. Dual-energy X-ray absorptiometry (DXA) is a rapid and non-invasive method for the measurement of fat mass, lean tissue mass (excluding bone), and bone mineral content in animals and humans. Its interest relies on the ability to obtain these measurements in vivo. Stomach emptying and immobilisation of the animal (with anaesthesia) during the process increase the measurement accuracy.

Individuals were allowed to fast for at least one hour, before being anaesthetised in an induction box containing 3% isoflurane in a 150 ml air flow (Univentor 410 anaesthesia unit (UniventorND, Zejtun, Malta)). Animals were kept anaesthetised using a rodent anaesthesia facemask, and the isoflurane concentration was lowered to 1.7%. Anaesthetized animals were placed under the X-ray beam of the dual-energy X-ray absorptiometry system (DXA, PIXImus, GE Lunar, GE Healthcare, WI, USA). Image acquisition for the DXA measurement lasted less than 5 minutes per animal. Animals were monitored and were returned to their cages only when consciousness was entirely recovered. When the deviation (in per cent) of the measurements was larger than 2%, the machine was recalibrated to ensure a standard deviation of fat percentage measurement lower than 0.3.

G. Additional analysis: Ankle width measurement

The bimalleolar ankle width of both rear legs was measured by the same examiner all along the experiment, following a standard procedure described in [18]. The vole was always presented to the measurer in the same position, left leg and right leg were measured with a calliper ruler

(Electronic Digital Caliper, Scala, Dettingen, Germany) and the mean measurement was computed. Individual identity explained the variation in ankle measurements ($F = 2.02$, $P < 0.001$) and based on 77 individuals measured after the enclosure period, the repeatability of the measurement was 0.74.

H. Measurement of reproductive success and paternity analysis

In the animal facility, the captured females were checked for parturition every day, and the litter size was assessed at birth [19]. At birth, each pup was individually marked, measured (head width and body mass) and sexed (by the help of visual cues and the length of anogenital distance) using a binocular microscope [20]. Paternity analysis was performed by genotyping the DNA from a small tissue sample taken from the tail tip of each pup at birth. DNA was extracted from these tissue samples and the adult ear biopsies, and individuals were genotyped at six microsatellite loci: 6G11, 10A11, 13G2, 15F7, 16E2, and 17E9 [21,22]. Paternity was assigned to the most likely male candidate with a confidence level of 95% using the software Cervus 3.0.7 while accounting for the known genotype of the mother [23,24]. All males released in the enclosure were considered as potential genitors regardless of whether they were trapped or not at the end of the experiment. The simulation was performed using 10000 cycles, 100% of candidate parents sampled, 98% of loci typed, a genotyping error rate of 1%, and no mismatches between offspring, neither with the known mother nor with the assigned father. Only one offspring with incomplete genotyping was not assigned paternity.

I. Details of the ELISA

We implemented an in-house enzyme-linked immunosorbent assay (ELISA) targeting *B. burgdorferi* s. l.-specific IgG antibodies using the protocol by Salo *et al.* [25], which we modified slightly. Before and after the enclosure period, a blood sample (75 µl) was taken from the retro-orbital sinus with a heparinised microcapillary and was placed in a heparin blood collection tube with separator gel (minicollect, Greiner bio one) and centrifuged (6000 rpm for 10 min). The resultant serum was frozen at -20 °C until used in the ELISA described hereinafter. Microtiter plates (Thermo Fisher Scientific) were coated with antigen from whole *B. afzelii* cells diluted in PBS (final concentration 10 µg/ml) at 37 °C overnight and washed three times with Aqua-Tween (H_2O, 0.05% Tween 20, Merck). The vole serum samples were diluted 1:100 in 1% bovine serum albumin (BSA, Serological Proteins Inc., Kankakee, USA) in PBS. The plates were incubated with the diluted serum samples at 37 °C for 1 h, washed three times as above, and incubated with HRP-conjugated goat anti-mouse IgG secondary antibody (final concentration 0.08 µg/ml, Pierce, Thermo Fisher Scientific) in PBS at 37 °C for 1 h. After the last three washes, ortho-phenylene-diamine (OPD, Kem- En-Tec Diagnostics A/S, Uppsala Sweden) substrate was added for 15 minutes before the reaction was stopped with 0.5 M H_2SO_4. The absorbance was measured at a wavelength of 492 nm with Multiskan EX spectro-photometer (Thermo Fisher Scientific). Samples were run in duplicate, and a positive control (serum from laboratory mouse infected with *B. burgdorferi* sensu stricto as confirmed by culture) was included in duplicate in each plate. Two types of negative controls were included in duplicate in each plate to control for nonspecific binding [26]: sera from uninfected laboratory mice, and blanks containing 1% BSA in PBS. The repeatability, assessed on 34 samples measured 4 to 6 times, was 0.99.

J. Details of the statistical analysis

All statistical analyses were carried out using the statistical software R version 3.1.1, and using the packages lme4, glmmADMB, MASS, sjPlot, secr, sp, and adehabitat. In the statistical analyses, the injection treatment (*Borrelia afzelii* vs. PBS) was used to define infection treatment (infected vs. uninfected). Most of the full models included the three-way interaction between infection, sex and density (Table S1). Model reduction was a step-down process, but the two experimentally manipulated factors, *B. afzelii* infection status of the bank voles (categorical: uninfected control versus infected; hereafter called "infection") and population density in the enclosure (categorical: low versus high; hereafter called "density"), were always included in the final models. When the three-way interaction was significant (as observed for the relative number of offspring, the relative number of partners, the home range perimeter and the home range surface, see Table S1), separate analyses were conducted for each sex to facilitate the interpretation of the interactions. When the three-way interaction was not statistically significant (ankle width, body mass, body fat proportion, survival, and breeding probability, see Table S1), it was removed from the full model, and the resultant simplified model was tested for statistical significance of the two-way interactions.

When included as a covariate, body mass and head width were centred to the mean observed value, separately for males and females. The enclosure was always modelled as a random effect.

1. Body mass and body fat content

Body mass and body fat proportion at the end of the study were modelled with a linear mixed model (LMM) with a Gaussian error distribution and a generalised linear mixed model (GLMM) with a beta error distribution, respectively. The fixed factors included infection, density, sex and

their two- and three-way interaction terms. Body mass before injection (BM) with *B. afzelii* or PBS was included as a covariate.

2. Survival and reproduction

Survival of bank voles in the enclosures and individual breeding probability are binary variables. For survival, individuals trapped at the end of the experiment were assigned a value of 1, whereas individuals not trapped were assigned a value of 0. For female breeding probability, individuals that did not give birth or that were not captured were assigned a value of 0, whereas females that gave birth were assigned a value of 1. For male breeding probability, individuals were assigned 0 or 1 depending on whether they sired zero offspring or at least one offspring according to the paternity test. All males introduced in the enclosures were included in the analyses of male reproductive success, regardless of whether they were trapped or not at the end of the study. These binary variables were modelled using a GLMM with a binomial error distribution and a logit link function. The fixed effects structure of the model of the breeding probability contained: infection, density, sex, BM or head width before injection (HW), and all two- and three-way interactions between infection, sex and BM or HW. For survival, the fixed structure of the model contained infection, density, sex, and all the two-way and three-way interactions as well as body mass as a covariate.

We used the paternity analyses and the monitoring of female litters to calculate two different response variables of reproductive success: (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 in an enclosure with which a given individual has successfully mated. These two proportional variables were modelled as a GLMM with a binomial error distribution and a logit link using penalised quasi-likelihood (which corrects for

overdispersion) as a function of infection, density, sex, and all two-way and three-way interactions. The BM or head width were included as covariates. Since the three-way interaction infection × density × sex was significant, separate analyses were conducted for each sex.

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 in a GLMM with a negative binomial distribution and log link.

3. Body mass and head width of the pups

The body mass and head width of the pups at birth were analysed using LMM with mother infection status, father infection status, density, their three-way interactions, sex of the pup and litter size in the fixed effects structure. The mother identity, the father identity, and the enclosure were included as random effects in each of these models.

4. Spacing behaviour

We used the space trapping data to calculate two different home range variables: (1) home range perimeter (m) and (2) home range surface (m^2) [27]. These two home range variables were analysed using LMM with infection, density, sex and all their two-way and three-way interactions in the fixed effects structure. Body mass before injection was included as a covariate. Since the three-way interaction was significant, separate analyses were conducted for each sex.

5. Clinical signs of infection

Infection with *B. burgdorferi* s. s. causes inflammation and increases the ankle width in some species and strains of rodents [28]. The post-infection ankle width was modelled using an LMM with the fixed effects infection, density, sex and all their two-way and three-way interactions.

The ankle width before infection was included as a covariate.

II. Additional analysis and additional results

A. Clinical signs

Infection and population density did not affect ankle width in infected individuals (LMM: $p = 0.02$, $p = 0.54$, see Table S5). Previous studies showed that *Mus musculus* mice infected with *B. burgdorferi* s. s. had significantly swollen ankle joints and detectable spirochetes from the ankle joints as early as two weeks after infection [25,29,30].

The body mass of male or female bank voles was not affected by *B. afzelii* infection or population density (see Tables S1 & S3). Likewise, there was no effect of *B. afzelii* infection or population density on an individual's body fat proportion (GLMM: $p > 0.60$, see Table S2).

B. Immune response

1. Method

We used LMM to model the *B. burgdorferi* s. l.-specific IgG-antibody response (assessed as the mean absorbance in the ELISA assay) in infected male bank voles as a function of male reproductive success, density and their interaction. Given that most of the females reproduced, the effect of reproduction on the level of the *B. burgdorferi* s. l.-specific IgG-antibody response in females was not estimated. Male reproductive success was coded as either a binary variable (no offspring, at least one offspring) or as a discrete variable (the number of offspring sired by each male). We further modelled the mean absorbance in the ELISA assay as a function of the head width, male reproductive success (binary) and their interaction. The mean absorbance of the positive controls was included as an offset in each model to account for variation between

ELISA plates and enclosures were modelled as a random effect.

2. Result: Immune response was affected by body size and reproduction in male bank voles

Analysis of the *B. burgdorferi* s. l.-specific IgG antibody response in infected males found that small males had similar levels of antibodies regardless of whether they reproduced or not, whereas, in large males, the individuals that reproduced were also the ones that mounted a higher immune response (LMM: $p = 0.03$, Fig S5, Table S7). When body mass was not accounted for, males had the same level of antibodies regardless of whether they reproduced or not. These findings suggest that large infected males have a lower probability of reproduction than small males, but when these large males reproduce, they are able to allocate energy to both reproduction and the immune response whereas small males that reproduce show lower immune capacity.

Supplementary tables

Table S1: Full models with three-way interactions

Response variable	Predictors	Intercept (SE)	Estimates (SE)	p-value	Random effect: enclosure; σ^2 (SD)	n
Body mass	Infection (inf)	25.56 (0.62)	-0.64 (0.79)	0.42	0.40 (0.63)	77
	Density (low)		0.12 (0.99)	0.90		
	Sex (male)		-1.91 (0.94)	**0.05**		
	BM		0.57 (0.11)	**<0.01**		
	Infection:sex		0.65 (1.30)	0.62		
	Sex:density		0.48 (1.50)	0.75		
	Infection:density		0.68 (1.30)	0.60		
	Infection:density:sex		-0.17 (2.07)	0.94		
Body fat proportion	Infection (inf)	-1.91 (0.06)	0.13 (0.09)	0.14	1.13e-07 (0.00)	77
	Density (low)		0.08 (0.10)	0.45		
	Sex (male)		0.20 (0.10)	0.05		
	BM		0.03 (0.01)	**0.04**		
	Infection:sex		-0.26 (0.15)	0.07		
	Sex:density		-0.15 (0.16)	0.36		
	Infection:density		-0.16 (0.15)	0.27		
	Infection:density:sex		0.16 (0.23)	0.49		
Survival	Infection (inf)	1.10 (0.52)	0.00 (0.73)	0.10	0.01 (0.09)	136
	Density (low)		-0.51(0.76)	0.50		
	Sex (male)		-1.50 (0.69)	**0.03**		
	BM		0.01 (0.08)	0.88		
	Infection:sex		0.20 (0.97)	0.84		
	Sex:density		0.91 (1.04)	0.38		
	Infection:density		-0.01(1.08)	0.99		
	Infection:density:sex		-0.49 (1.47)	0.74		
Breeding probability	Infection (inf)	1.18 (0.43)	-0.02 (0.55)	0.98	0.00 (0.00)	136
	Density (low)		-1.02 (0.39)	**0.01**		
	Sex (male)		0.10 (0.56)	0.86		
	HW		-1.50 (1.22)	0.22		
	Infection:HW		-0.21 (1.75)	0.90		
	Infection:sex		-0.98 (0.77)	0.21		
	Sex:HW		3.72 (1.67)	**0.03**		
	Infection:HW:sex		-3.29 (2.30)	0.15		
Breeding probability	Infection (inf)	1.17 (0.42)	-0.07 (0.55)	0.90	0.00 (0.00)	136
	Density (low)		-0.94 (0.39)	**0.02**		
	Sex (male)		0.07 (0.56)	0.89		
	BM		-0.14 (0.17)	0.42		
	Infection:BM		-0.27 (0.27)	0.33		
	Infection:sex		-0.92 (0.77)	0.24		
	Sex:BM		0.48 (0.25)	**0.05**		
	Infection:BM:sex		-0.14 (0.35)	0.68		

Relative number of partners	Infection (inf)	-1.85 (0.24)	0.01 (0.34)	0.97	$5.05*10^{-5}$ (0.01)	136
	Density (low)		0.20 (0.46)	0.67		
	Sex (male)		0.05 (0.04)	0.30		
	BM		0.31 (0.35)	0.38		
	Infection:sex		0.09 (0.50)	0.85		
	Sex:density		1.10 (0.63)	0.09		
	Infection:density		0.21 (0.63)	0.73		
	Infection:density:sex		-2.17 (0.98)	0.03		
Relative number of offspring	Infection (inf)	-1.97 (0.26)	0.05 (0.36)	0.89	$2.85* 10^{-5}$ (0.01)	136
	Density (low)		1.00 (0.42)	**0.04**		
	Sex (male)		0.07 (0.04)	0.14		
	BM		-0.09 (0.37)	0.79		
	Infection:sex		0.19 (0.51)	0.71		
	Sex:density		0.78 (0.59)	0.19		
	Infection:density		-0.30 (0.61)	0.63		
	Infection:density:sex		-2.39 (0.99)	**0.02**		
Ankle width	Infection (inf)	2.73 (0.17)	0.02 (0.04)	0.57	0.00 (0.00)	77
	Density (low)		0.01 (0.05)	0.78		
	Sex (male)		0.13 (0.05)	**0.01**		
	Ankle To		0.04 (0.06)	0.56		
	Infection:sex		-0.05 (0.07)	0.43		
	Sex:density		-0.11 (0.07)	0.15		
	Infection:density		0.00 (0.06)	0.97		
	Infection:density:sex		0.08 (0.10)	0.46		
Home range perimeter	Infection (inf)	100.46 (43.04)	-10.87 (57.38)	0.85	1123 (33.52)	70
	Density (low)		26.38 (73.73)	0.72		
	Sex (male)		128.32 (66.77)	0.06		
	BM		6.73 (8.16)	0.41		
	Infection:density		19.65 (98.47)	0.84		
	Infection:sex		144.75 (92.56)	0.12		
	Density:sex		179.37 (111.79)	0.11		
	Infection:density:sex		-361.07 (154.23)	**0.02**		
Home range surface	Infection (inf)	147.25 (51.99)	15.37 (68.95)	0.82	2457 (49.56)	70
	Density (low)		67.70 (88.93)	0.45		
	Sex (male)		222.33 (80.24)	**<0.01**		
	BM		10.94 (9.81)	0.27		
	Infection:density		30.90 (118.39)	0.79		
	Infection:sex		137.51 (111.21)	0.22		
	Density:sex		374.81 (134.44)	**<0.01**		
	Infection:density:sex		-648.89 (184.43)	**<0.01**		

BM: centred value of body mass before injection; HW: centred value of head width before injection, Ankle To: centred value of join width before injection, inf: infected group; low: low population density. σ^2 is the variance attributable to the random effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in bold.**

306 **Table S2: Selected final models for fitness and spacing behaviour of male bank voles**

307

Response variables	Predictors	Intercept (SE)	Estimates (SE)	t-value	p-value	Random effect: enclosure, σ^2 (SD)	n
Relative number of offspring	Infection (inf)	-2.08 (0.31)	0.20 (0.42)	0.49	0.63	$1.19*10^{-9}$ ($3.46*10^{-5}$)	68
	Density (low)		1.76 (0.47)	3.79	**<0.01**		
	BM		0.16 (0.07)	2.31	**0.03**		
	Infection:Density		-2.72 (0.89)	-3.06	**<0.01**		
Relative number of partners	Infection (inf)	-1.52 (0.28)	0.07 (0.39)	0.18	0.86	$1.11*10^{-9}$ ($3.33*10^{-5}$)	68
	Density (low)		1.24 (0.48)	2.59	**0.03**		
	BM		0.15 (0.06)	2.31	**0.03**		
	Infection:Density		- 1.95 (0.82)	-2.38	**0.02**		
Home range perimeter	Infection (inf)	233.16 (75.70)	129.35 (99.18)	1.30	0.21	2646 (51.44)	27
	Density (low)		191.11 (122.09)	1.57	0.14		
	Infection:Density		-318.17 (163.55)	-1.95	0.07		
Home range surface	Infection (inf)	378.18 (78.24)	146.38 (107.54)	1.36	0.19	0.00 (0.00)	27
	Density (low)		429.71 (126.16)	**3.41**	**<0.01**		
	Infection:Density		-594.50 (176.51)	**3.37**	**<0.01**		

308

309 BM: centred value of body mass before injection, inf: infected group; low: low population

310 density; σ^2 is the variance attributable to the random effect, SD is the standard deviation, SE is

311 the standard error. **Significant effects are marked in bold.**

312 **Table S3: Selected final models for fitness and spacing behaviour of female bank voles**

313

Response variables	Predictors	Intercept (SE)	Estimates (SE)	t-value	z-value	p-value	Random effect: enclosure, σ2 (SD)	n
Relative number of offspring	Infection (inf) Density (low)	-1.91 (0.19)	-0.06 (0.24) 0.85 (0.25)	-0.26 **3.35**		0.79 **<0.01**	$2.31*10^{-5}$ (0.00)	68
Relative number of partners	Infection (inf) Density (low)	-1.87 (0.20)	0.07 (0.25) 0.31 (0.28)	0.28 1.15		0.29 1.13	$5.04*10^{-5}$ (0.01)	68
Parturition delay	Infection (inf) Density (low)	1.70 (0.18)	-0.75(0.25) -0.28 (0.26)		-3.00 -1.04	**<0.01** 0.30	0.00 (0.00)	45
Home range perimeter	Infection (inf) Density (low) BM	98.74 (23.81)	0.47 (29.91) 39.81 (31.38) 19.56 (7.30)	0.02 1.27 2.68		0.99 0.21 **0.01**	0.00 (0.00)	43
Home range surface	Infection (inf) Density (low) BM	143.17 (36.78)	27.51 (46.20) 90.42 (48.46) 23.82 (11.27)	0.59 2.11 1.87		0.56 0.07 **0.04**	0.00 (0.00)	43

314

315 inf: infected group; low: low population density; σ2 is the variance attributable to the random

316 effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in**

317 **bold.**

Table S4: Selected final models for survival and breeding probability in adult bank voles (males and females combined)

Response variables	Predictors	Intercept (SE)	Estimates (SE)	z-value	p-value	Random effect: enclosure, σ2 (SD)	n
Survival	Infection (inf)	0.94 (0.36)	-0.00 (0.36)	0.00	1.00	0.00 (0.03)	136
	Density (low)		-1.49 (0.37)	-0.41	0.68		
	Sex (male)		-1.11 (0.36)	-3.06	**<0.01**		
Breeding probability	Infection (inf)	1.42 (0.39)	-0.52 (0.37)	-1.39	0.16	0.00 (0.00)	136
	Density (low)		-0.96 (0.38)	-2.53	**0.01**		
	Sex (male)		-0.46 (0.37)	-1.23	0.22		
	HW		0.66 (0.77)	0.86	0.39		
	Infection:HW		-2.02 (1.08)	-1.68	0.06		
Breeding probability	Infection (inf)	1.40 (0.39)	-0.53 (0.38)	-1.39	0.16	0.00 (0.00)	136
	Density (low)		-0.93 (0.38)	-2.42	**0.02**		
	Sex (male)		-0.42 (0.38)	-1.10	0.27		
	BM		-0.11 (0.15)	-0.71	0.48		
	Infection:BM		-0.33 (0.17)	-2.00	**0.05**		
	Sex:BM		0.40 (0.17)	2.27	**0.02**		

BM: centred value of body mass before injection, HW: centred value of head width before injection, inf: infected group, low: low population density; σ2 is the variance attributable to the random effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in bold.**

Table S5: Selected final models for body mass, body fat proportion and ankle width in adult bank voles (males and females combined)

Response variables	Predictors	Intercept (SE)	Estimates (SE)	z-value or t-value	p-value	Random effect: enclosure, σ2 (SD)	n
Body mass	Infection (inf)	22.30 (0.58)	-0.17 (0.51)	-0.33	0.74	0.69 (0.83)	77
	Density (low)		0.58 (0.71)	0.82	0.44		
	Sex (male)		1.47 (0.53)	-2.78	**0.01**		
	BM		0.58 (0.11)	5.15	**<0.01**		
Body fat proportion	Infection (inf)	-1.80 (0.05)	-0.00 (0.06)	-0.08	0.94	$1.93*10^{-7}$ (0.00)	77
	Density (low)		-0.03 (0.06)	-0.51	0.61		
	BM		0.02 (0.01)	2.09	**0.04**		
Ankle width	Infection (inf)	2.84 (0.02)	0.01 (0.03)	0.56	0.58	0.00 (0.00)	77
	Density (low)		-0.01 (0.03)	-0.36	0.72		
	Sex (male)		0.07 (0.03)	2.60	**0.01**		

BM: centred value of body mass before injection, inf: infected group, low: low population density; σ2 is the variance attributable to the random effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in bold.**

Table S6: Selected final models for the body mass and head width of pup at birth

Response variable	Predictors	Intercept (SD)	Estimates (SD)	t-value	p-value	Random effect:, σ2 (SD)	n
Pup body mass at birth	Mother (inf)	2.27 (0.11)	-0.08 (0.05)	-1.60	0.12	Mother: 0.02 (0.14) Father: 0.00 (0.00) Enclosure: 0.002 (0.04)	224
	Father (inf)		-0.01 (0.03)	-0.44	0.67		
	Density (low)		0.06 (0.06)	1.09	0.30		
	Offspring sex (male)		0.08 (0.02)	3.40	**<0.01**		
	Litter size		-0.08 (0.02)	-3.89	**<0.01**		
Pup head width at birth	Mother (inf)	51.90 (1.12)	-0.43 (0.49)	-0.87	0.39	Mother: 2.24 (1.50) Father: 0.00 (0.00) Enclosure: 0.05 (0.24)	224
	Father (inf)		0.25 (0.30)	0.81	0.42		
	Density (low)		1.17 (0.54)	2.15	0.05		
	Offspring sex (male)		0.73 (0.19)	3.79	**<0.01**		
	Litter size		-0.55 (0.21)	-2.68	**0.01**		

inf: infected group for the mother or the father; low: low population density, σ2 is the variance attributable to random effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in bold.**

Table S7: Selected final models for the *B. burgdorferi* s. l.-specific IgG antibody response of the subset of infected male bank voles (mean absorbance of the positive control was used as an offset in all models)

Response variable	Predictors	Intercept (SD)	Estimates (SD)	t-value	p-value	Random effect: enclosure, σ2 (SD)	n
ELISA mean absorbance	Reproduction (yes)	-0.48 (0.10)	0.04 (0.14)	0.27	0.80	0.02 (0.14)	15
	Density (low)		0.11 (0.12)	0.97	0.36		
	Density*Reproduction		-0.24 (0.18)	-1.36	0.23		
ELISA mean absorbance	N_offspring	-0.42 (0.1)	0.00 (0.01)	0.21	0.84	0.02 (0.14)	15
	Density (low)		-0.02 (0.14)	-0.15	0.88		
	Density*N_offspring		-0.05 (0.04)	-1.13	0.31		
ELISA mean absorbance	HW	-0.50 (0.06)	-0.35 (0.18)	-1.98	0.07	0.00 (0.03)	15
	Reproduction (yes)		0.15 (0.08)	1.73	0.11		
	HW*Reproduction		0.66 (0.26)	**2.53**	**0.03**		

HW: centred value of head width before injection, yes: male produced at least one offspring; low: low population density; σ2 is the variance attributable to random effect, SD is the standard deviation, SE is the standard error. **Significant effects are marked in bold.**

Supplementary figures

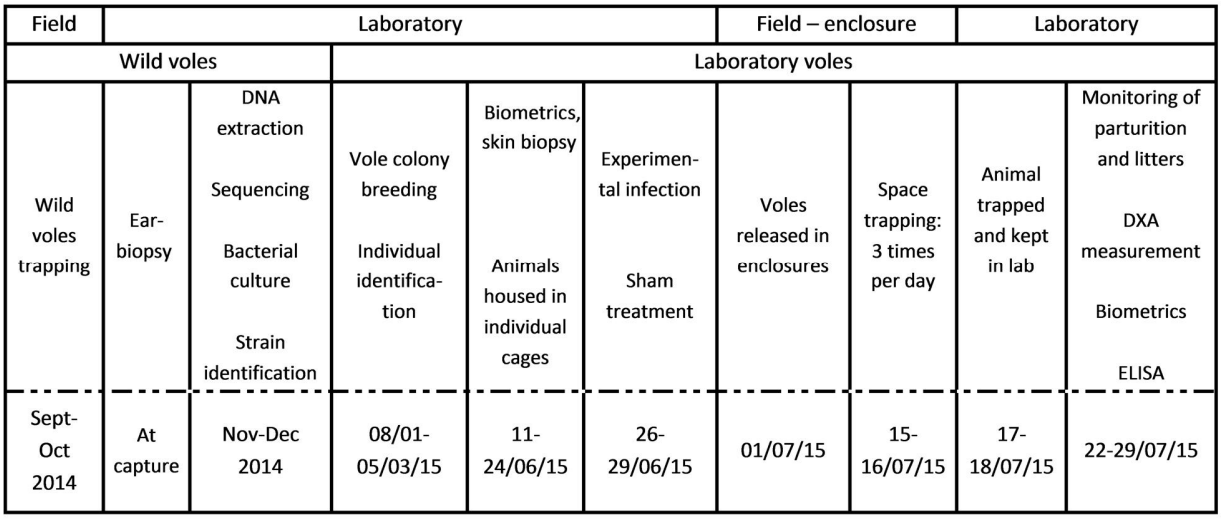


Figure S1. Schematic representation of the experimental procedure.

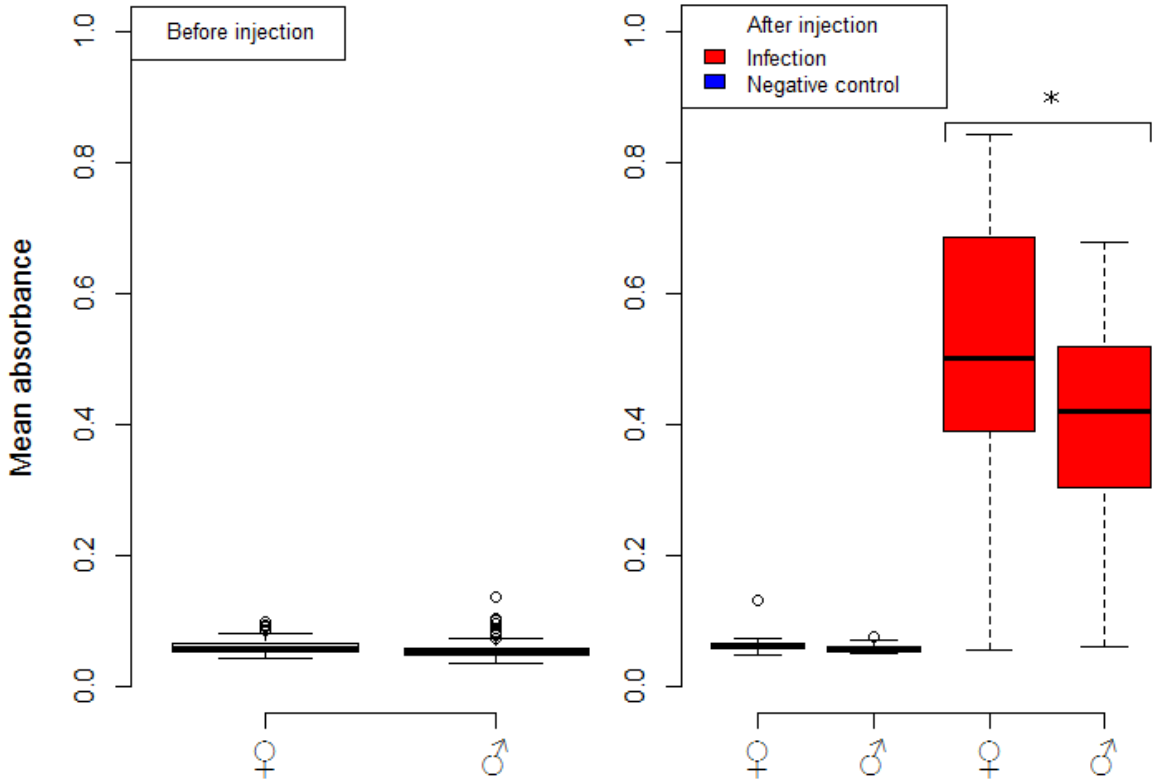
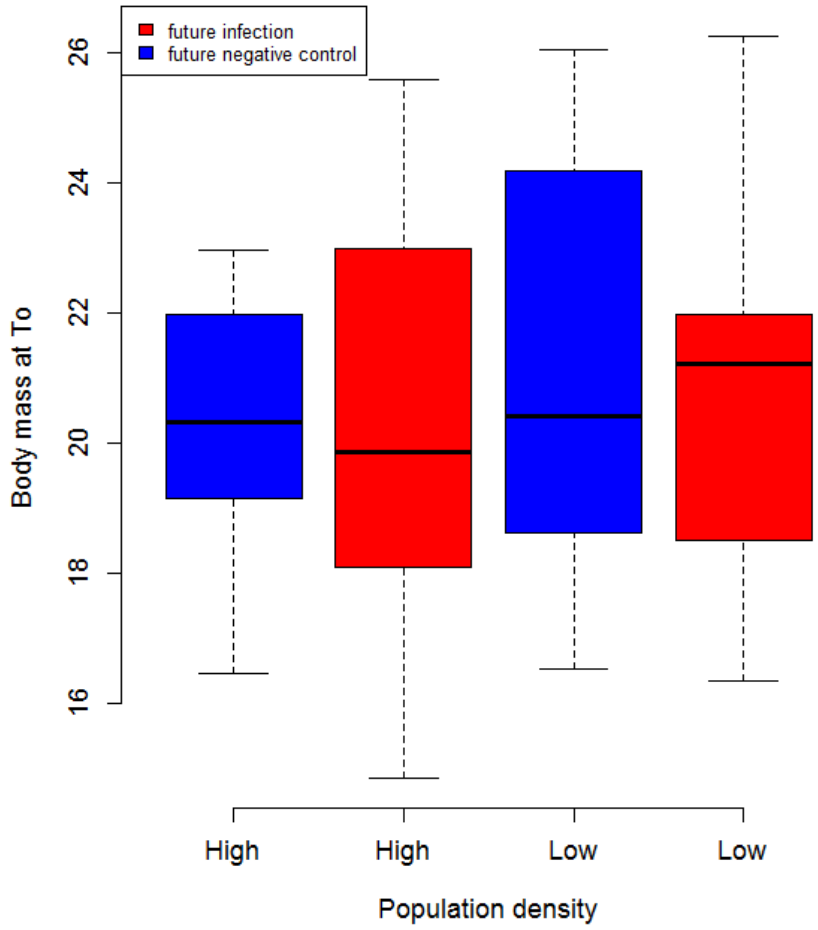


Figure S2. The bank voles in the infection group developed a strong *B. burgdorferi* s. l.-specific IgG antibody response compared to the control group. The strength of the *B. burgdorferi* s. l.-specific IgG antibody response was measured as the absorbance on an ELISA test. Mean absorbance of plasma samples before and after injection of *B. afzelii* are shown. The mean absorbance in the ELISA was significantly explained by infection (ANOVA: Fdf1, df2=3.02, $p < 0.001$, Fig. S2), but not by sex (ANOVA: Fdf1, df2 = 0.04, Df = 1, $p = 0.20$).



359

360 Figure S3. Male body mass was similar among the four combinations of infection

361 treatment and population density before injection with *B. afzelii* or with PBS.

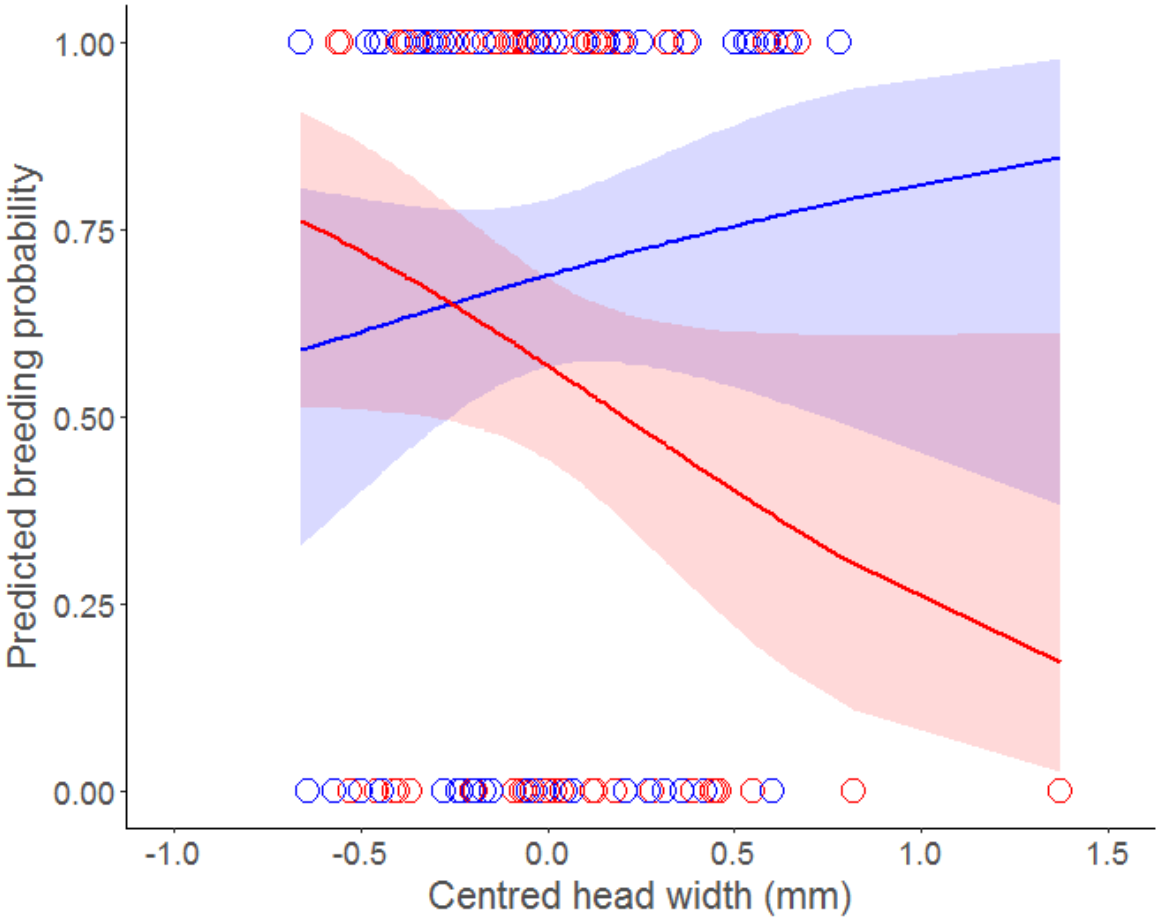


Figure S4. Predicted probability of reproduction for a bank vole explained by infection (uninfected individuals in blue, infected individuals in red) and initial head width, as shown in table S4 (p=0.06). The observed values are shown with open circles (n = 136 bank voles).

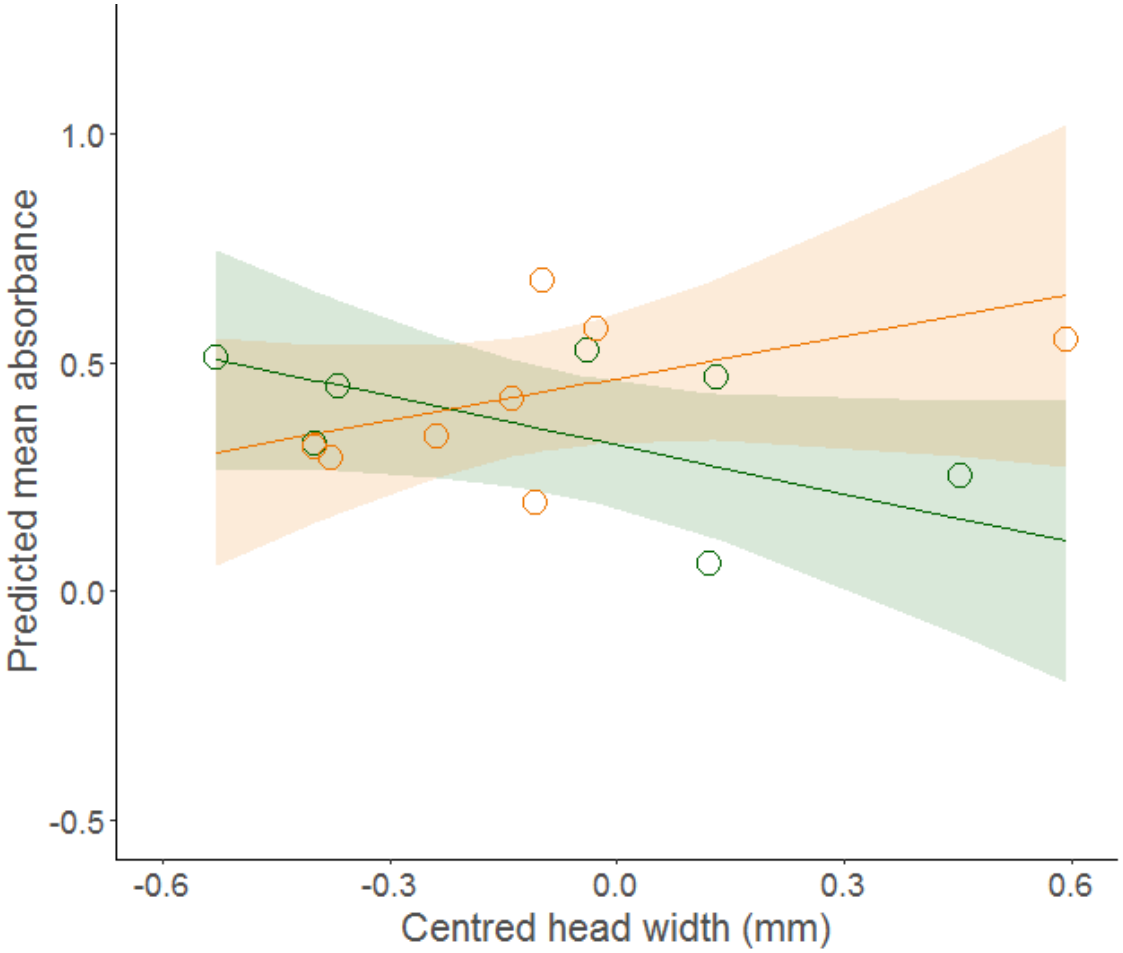


Figure S5. Predicted *B. burgdorferi* s. l.-specific IgG antibody levels in infected male bank voles depends on the interaction between reproductive status (individuals that reproduced in orange, individuals that did not reproduce in green) and body size. Body size is measured as the initial head width. For males that reproduced (n = 8), the strength of the *B. burgdorferi* s. l.-specific IgG antibody response increased with body size. For males that did not reproduce (n = 7), the strength of the *B. burgdorferi* s. l.-specific IgG antibody response was not influenced by body size. The observed values are showed with open circles (n = 15 infected male bank voles). For statistics, see Table S7.

References

1. Hofmeister EK, Childs JE. 1995 Analysis of Borrelia burgdorferi sequentially isolated from Peromyscus leucopus captured at a Lyme disease enzootic site. *J. Infect. Dis.* **172**, 462–469.

2. Wodecka B, Leońska A, Skotarczak B. 2010 A comparative analysis of molecular markers for the detection and identification of Borrelia spirochaetes in Ixodes ricinus. *J. Med. Microbiol.* **59**, 309–314. (doi:10.1099/jmm.0.013508-0)

3. 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.

4. Durand J, Jacquet M, Paillard L, Rais O, Gern L, Voordouw J. 2015 Cross-immunity and community structure of a multiple-strain pathogen in the tick vector. *Appl. Environ. Microbiol.* **81**, 7740–7752. (doi:10.1128/AEM.02296-15.Editor)

5. Wang I-N, Dykhuizen DE, Qiu W, Dunn JJ, Bosler EM, Luft BJ. 1999 Genetic diversity of ospC in a local population of Borrelia burgdorferi sensu stricto. *Genetics* **151**, 15–30.

6. Bunikis J, Garpmo U, Tsao J, Berglund J, Fish D, Barbour AG. 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. (doi:10.1099/mic.0.26944-0)

7. Bey RF, Loken KI, Wu CC, Lin TL. 1995 Experimental infection of the red-backed vole (Clethrionomys gapperi) with Borrelia burgdorferi. *J. Wildl. Dis.* **31**, 428–431.

8. McLean RG, Ubico SR, Cooksey LM. 1993 Experimental infection of the eastern chipmunk (Tamias striatus) with the Lyme disease spirochete (Borrelia burgdorferi). *J Wildl Dis* **29**, 527–532.

9. Baum E, Hue F, Barbour AG. 2012 Experimental infections of the reservoir species Peromyscus leucopus with diverse strains of Borrelia burgdorferi, a Lyme disease agent. *MBio* **3**. (doi:10.1128/mBio.00434-12)

10. Johnson RC, Kodner CL, Russell ME. 1986 Vaccination of hamsters against experimental infection with Borrelia burgdorferi. *Zentralbl Bakteriol Mikrobiol Hyg A* **263**, 45–48.

11. Moody KD, Terwilliger GA, Hansen GM, Barthold SW. 1994 Experimental Borrelia burgdorferi infection in Peromyscus leucopus. *J. Wildl. Dis.* **30**, 155–61. (doi:10.7589/0090-3558-30.2.155)

12. Oksanen TA, Jokinen I, Koskela E, Mappes T, Vilpas H. 2003 Manipulation of offspring number and size: Benefits of large body size at birth depend upon the rearing environment. *J. Anim. Ecol.* **72**, 321–330. (doi:10.1046/j.1365-2656.2003.00703.x)

13. Voutilainen L, Kallio ER, Niemimaa J, Vapalahti O, Henttonen H. 2016 Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. *Sci. Rep.* **6**. (doi:10.1038/srep21323)

14. Koivula M, Koskela E, Mappes T, Oksanen T a. 2003 Cost of reproduction in the wild: Manipulation of reproductive effort in the bank vole. *Ecology* **84**, 398–405.

15. Bujalska G. 1985 Fluctuations in an island bank vole population in the light of the study on its organization. *Acta Theriol. (Warsz).* **30**, 3–49.

16. Calenge C. 2006 The package ‘adehabitat’ for the R software: A tool for the analysis of space and habitat use by animals. *Ecol. Modell.* **197**, 516–519. (doi:10.1016/j.ecolmodel.2006.03.017)

17. Kenward RE, Clarke RT, Hodder KH, Walls SS. 2001 Density and linkage estimators of home range: nearest-neighbor clustering defines multinuclear cores. *Ecology* **82**, 1905–1920. (doi:10.1890/0012-9658(2001)082[1905:DALEOH]2.0.CO;2)

18. Hill JA *et al.* 2008 Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. *J. Exp. Med.* **205**, 967–979. (doi:10.1084/jem.20072051)

19. Mappes T, Koskela E, Ylonen H. 1995 Reproductive costs and litter size in the bank vole. *Proc. R. Soc. B-Biological Sci.* **261**, 19–24. (doi:10.1098/rspb.1995.0111)

20. Koskela E, Mappes T, Niskanen T, Rutkowska J. 2009 Maternal investment in relation to sex ratio and offspring number in a small mammal - A case for Trivers and Willard theory? *J. Anim. Ecol.* **78**, 1007–1014. (doi:10.1111/j.1365-2656.2009.01574.x)

21. Gockel J, Harr B, Schlötterer C, Arnolds W, Gerlach G, Tautz D. 1997 Isolation and characterization of microsatellite loci from Apodemus flavicollis (rodentia , muridae) and Clethrionomys glareolus (rodentia , cricetidae). *Mol. Ecol.* **6**, 597–599. (doi:10.1046/j.1365-294X.1997.00222.x)

22. Rikalainen K, Grapputo A, Knott E, Koskela E, Mappes T. 2008 A large panel of novel microsatellite markers for the bank vole (Myodes glareolus). *Mol. Ecol. Resour.* **8**, 1164–1168. (doi:10.1111/j.1755-0998.2008.02228.x)

441 23. Mills SC, Grapputo A, Koskela E, Mappes T. 2007 Quantitative measure of sexual
442 selection with respect to the operational sex ratio: a comparison of selection indices. *Proc.*
443 *R. Soc. Biol. Sci. Ser. B* **274**, 143–50. (doi:10.1098/rspb.2006.3639)

444 24. Mills S, Grapputo A, Jokinen I, Koskela E, Mappes T, Oksanen TA, Poikonen T. 2009
445 Testosterone-mediated effects on fitness related phenotypic traits and fitness. *Am. Nat.*
446 **173**, 475–487. (doi:10.1086/597222)

447 25. Salo J, Jaatinen A, Soderstrom M, Viljanen MK, Hytonen J. 2015 Decorin binding
448 proteins of Borrelia burgdorferi promote arthritis development and joint specific post-
449 treatment DNA persistence in mice. *PLoS One* **10**, 1–17.
450 (doi:10.1371/journal.pone.0121512)

451 26. Crowther JR. 2001 *The ELISA guidebook.* Human Pres. New Jersey.
452 (doi:10.1134/S000629790909017X)

453 27. Jonsson P, Koskela E, Mappes T. 2000 Does risk of predation by mammalian predators
454 affect the spacing behaviour of rodents? Two large-scale experiments. *Oecologia* **122**,
455 487–492. (doi:10.1007/s004420050970)

456 28. Wooten RM, Weis JJ. 2001 Host-pathogen interactions promoting inflammatory lyme
457 arthritis: Use of mouse models for dissection of disease processes. *Curr. Opin. Microbiol.*
458 **4**, 274–279. (doi:10.1016/S1369-5274(00)00202-2)

459 29. Wooten RM, Ma Y, Yoder RA, Brown JP, Weis JH, Zachary JF, Kirschning CJ, Weis JJ.
460 2002 Toll-like receptor 2 is required for innate, but not acquired, host defense to Borrelia
461 burgdorferi. *J. Immunol.* **168**, 348–355. (doi:10.4049/jimmunol.168.1.348)

30. Ma Y, Seiler KP, Eichwald EJ, Weis JH, Teuscher C, Weis JJ. 1998 Distinct characteristics of resistance to Borrelia burgdorferi induced arthritis in C57BL/6N mice. *Infect. Immun.* **66**, 161–168.