Rapid change in parasite infection traits over the course of an epidemic in a wild host-parasite population

Stuart K.J.R. Auld[1,2*], Philip J. Wilson[1] and Tom J. Little[1]

[1]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, UK

[2]Present address: School of Natural Sciences, University of Stirling, Stirling, UK

*Correspondence: School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, Scotland, UK.
Email: s.k.auld@stir.ac.uk; Tel: +44 (0)1786 467757;
Fax: +44 (0)1786 467843.

Abstract

By combining a field study with controlled laboratory experimentation, we examined how infection traits of the sterilizing bacterium, *Pasteuria ramosa*, changed over the course of a growing season in a natural population of its crustacean host *Daphnia magna*. The number of parasite transmission spores per infected host increased ten-fold over the course of the season, concomitant with a decline in the density of infected hosts. Plausible explanations for this variation include changes in environmental conditions, changes in host quality, or that parasite migration or natural selection caused a genetic change in the parasite population. We sought to distinguish some of these possibilities in a laboratory experiment. Thus, we preserved field-collected parasite spores throughout the season, and later exposed a set of hosts to a fixed dose of these spores under controlled laboratory conditions. Parasites collected late in the season were more infectious and grew more rapidly than parasites collected early in the season. This result is compatible with the hypothesis that the observed increase in infectivity in the field was due to genetic change, i.e., evolution in the *P. ramosa* population.

Introduction

Parasites and pathogen populations can change dramatically over very short time periods, as evidenced by the evolution of drug resistance (Marchese et al. 2000), the emergence of vaccine escape mutants (Bangham et al. 1999), the evolution of infectivity (the capacity to infect), the rate of within-host proliferation and the degree to which parasites stimulate the host's immune system (Paterson and Barber 2007). Shifts in infection traits may be due to genetic change in the parasite population, though this will not always be the case. For example, infection-related traits may change when the quality of the host as a resource changes due to shifts in host food quantity (Krist et al. 2004, Vale et al. 2011) and quality (Hall et al. 2009), or when there are changes in environmental factors such as temperature (Blanford et al. 2003, Vale et al. 2011).

We used a model system, the crustacean *Daphnia magna* and its sterilizing bacterial parasite *Pasteuria ramosa*, to both document an intriguing pattern of evolution in the field, and probe for its cause. The *Daphnia-Pasteuria* system has been used extensively to explore how various genetic and non-genetic phenomena affect the incidence and severity of parasitism (Auld et al. 2012a, Auld et al. 2012b, Auld et al. 2010, Duneau et al. 2011, Ebert 2008, Luijckx et al. 2011). Another laboratory study of this system has also provided compelling support for key predictions from the evolution of virulence theory, specifically that parasite virulence is traded-off with transmission potential (Jensen et al. 2006). These *P. ramosa* infection traits have been examined under controlled laboratory conditions and the relationship between within-host replication and virulence has not been much linked to natural epidemiological and disease severity patterns.

We studied changes in parasite prevalence (the proportion of infected hosts),

infection intensities (the number of parasites growing within each infected host) and the number of haemocytes circulating in the host across a growing season in a natural population. These parameters were associated with host population densities. To disentangle whether any observed changes in the field were due to changes in the parasite population or to changes in the complement of host genotypes, we exposed, under common garden conditions in the laboratory, a set of standard (i.e., reference) host genotypes to parasites collected (and then stored frozen) from different times during the field season. Thus we combined observations of parasite change in the field with an experiment that could shed light on possible causes of that change.

Materials and methods

Study organisms

Daphnia magna is a cyclically parthenogenetic freshwater planktonic crustacean that inhabits shallow freshwater ponds. *Daphnia* are frequently exposed to and infected with the sterilizing microparasite, *Pasteuria ramosa* (Ebert 2008). *Pasteuria ramosa* is a spore-forming bacterium that is transmitted horizontally from the corpses of previously infected hosts (Ebert et al. 1996). *Daphnia* take in *P. ramosa* transmission spores along with their food, and once in the host, the *P. ramosa* spores go through a 10-20 day developmental process, resulting in millions of transmission spores that are released upon host death. The process of parasite development and reproduction uses up resources that would otherwise be used for host reproduction, and *Daphnia* are almost always sterilised as a direct result of infection with *P. ramosa*. *Pasteuria*-infected *Daphnia* can be easily identified by eye: they have obvious red bacterial growth in their haemolymph, and are usually larger and lack developed ovaries and

eggs in their brood chamber. Whilst *P. ramosa* is known to infect many *Daphnia* and other cladoceran species (Auld et al. 2012c, Duffy et al. 2010, Ebert 2005), in this population *D. magna* is the only available host and is therefore the key agent in shaping *P. ramosa* infection traits.

The *Daphnia-Pasteuria* model has been used in many studies of parasite fitness because infection status is easily determined and transmission stages (henceforth transmission spores) are only released on the death of the host (Ebert et al. 1996). Good estimates of both parasite lifetime reproductive success and transmission potential can therefore be obtained by counting the number of transmission spores from infected hosts (Jensen et al. 2006), and *P. ramosa* spores remain infectious after being frozen at -20˚C (King et al. 2013). Also, the number of circulating haemocytes in the host rapidly increases soon after exposure to infectious *P. ramosa* spores (i.e., there is a cellular response: Auld et al. 2012a, Auld et al. 2012b, Auld et al. 2010), and baseline haemocyte number is greater in *P. ramosa*-infected as opposed to healthy hosts (Auld et al. 2012b).

Field haemocyte and parasite spore counts

Daphnia magna were sampled from three fixed points in a pond at Kaimes Farm, Leitholm, Scottish Borders (2˚20.43'W, 55˚42.15'N) twice per month between April and November 2010. This pond is approximately 500 m from the pond surveyed in a previous field study (Auld et al. 2012b). The pond contains other *Daphnia* species, but *P. ramosa* has only been observed to infect *D. magna.* Adult *D. magna* were collected by sweeping a net with an opening of $0.063m^2$ through one metre of pond water; they were then grouped according to infection status. Hosts from each grouping were placed five at a time in a cell extraction chamber with 4.0 µl of ice-

cold anticoagulant buffer (98 mM NaOH, 186 mM NaCl, 17 mM EDTA and 41 mM citric acid, pH adjusted to 4.5: Lavine et al. 2005) and their hearts were pierced using a 25-gauge needle (BD Microlance, Drogheda, Ireland), causing haemolymph to pool into the buffer. This haemolymph-buffer solution was then transferred into 0.5 ml Eppendorf tubes and placed on ice for the hour-long journey back to the laboratory. Cadavers were kept into 1.5 ml Eppendorf tubes.

In the laboratory, each of the haemolymph samples was mixed thoroughly and 2 μl were placed in a fertility counting chamber (0.001 mm^2 × 0.100 mm depth, Hawksley, Lancing, Sussex, UK). The number of haemocytes per microlitre of haemolymph-buffer solution was then determined. The cadavers were homogenized in 500 μl of ddH_2O, and 8 μl were placed in a Neubauer (Improved) counting chamber (0.0025 mm^2 × 0.1 mm depth), and the number of *P. ramosa* transmission spores (an estimate of parasite fitness) was determined. These spore solutions were then frozen at -20ºC.

At each sampling location, water temperature was measured using a digital field thermometer (HANNA instruments HI93510). The population density of *Daphnia* was estimated by counting the number of infected adults, healthy adults and juveniles. *Pasteuria ramosa* infection was assessed in the adult portion of all subsamples. Infection was usually assessed by eye, but in the occasional ambiguous case, individuals were crushed under a glass coverslip on a microscope slide, and then examined under a transmission microscope for the presence of *P. ramosa* spores.

Experimental setup

We performed two experiments. The first experiment was designed to test if (1) the ability of *P. ramosa* to infect, (2) its reproductive success in infected hosts and (3) its

147 ability to elicit a host cellular response changed over the course of the season. The
148 second experiment was designed to test whether the parasite-mediated mortality (a
149 measure of virulence that strongly influences *P. ramosa* life-history) depended on
150 whether *Daphnia* were infected with *P. ramosa* collected from the beginning or the
151 end of the season.

152 A test set of four standard *Daphnia* genotypes (named KA40, KA53, KA62
153 and KA81) were maintained as independent replicates. These host genotypes were
154 chosen because they vary in susceptibility to *P. ramosa* spores from this study
155 population. All four host genotypes were used in the first experiment, and KA53 and
156 KA62 were used in the second experiment. These genotypes were founded from
157 laboratory-hatched ephippia collected from a local pond (500m from the current study
158 site; see Auld et al. 2012b) and kept in the laboratory in a state of clonal reproduction
159 for three generations to minimize variation in condition. Hosts were kept in groups of
160 five in jars containing 200 ml of artificial medium (Kluttgen et al. 1994) and fed 5.0
161 ABS of chemostat-grown *Chlorella vulgaris* algal cells per day (ABS is the optical
162 absorbance of 650 nm white light by the *C. vulgaris* culture; 5.0 ABS is an abundance
163 of algal food). The jars were incubated at 20°C on a 12L:12D light cycle, and their
164 medium was changed three times per week, and after the *Daphnia* had offspring.
165 Second clutch neonates formed the experimental replicates in each of the two
166 experiments.

167 The parasite spores used were from samples collected during the field study on
168 June 8th, July 6th, August 3rd, August 17th, September 2nd and September 16th 2010.
169 These are from the same samples as spores used for the spore counts shown in Fig.
170 1B, and were frozen (at -20ºC) within three hours of collection. On the day of
171 experimentation, the spore samples were defrosted. Six consolidated spore solutions

(one for each date) were then made; they consisted of equal numbers of spores from each replicate sample collected on that specific date. Spore solutions were then diluted with ddH_2O until each final solution was at a concentration of 1×10^6 spores per ml.

Experiment 1

Each experimental replicate consisted of five *Daphnia* in 200 mL of artificial media. Replicates were divided between seven treatments: they were either controls, or were exposed to one of the six parasite spore solutions. There were 12 replicates per parasite treatment, per genotype and thus a total of 336 replicates.

Experimental replicates were kept in the same conditions as maternal generations until at least three of the five *Daphnia* deposited eggs in their brood pouch, at which point they were ready for parasite exposure. Parasite treatments were as follows: for each replicate, the five adult *Daphnia* were placed in the well of a 24-well plate (Costar, Corning Inc., NY, USA). Replicates assigned to the parasite-exposed treatments then received 5×10^4 *P. ramosa* spores (50 μl), and control replicates received an identical volume of homogenized healthy *Daphnia* as a placebo.

Treatment exposure lasted for five hours, after which the *Daphnia* were removed from the cell plate and washed in artificial medium. Four of the five hosts in each replicate were dried on a paper towel and then placed on a glass Petri dish. Their hearts were pierced with a 25 gauge needle (BD Microlance, Drogheda, Ireland), and from each of the four *Daphnia,* 1.0 μl of haemolymph was pipetted and mixed with 4 μl of anticoagulant buffer. Haemocytes were then counted using methodology described earlier. It is important to note that this measure of host cellular immune

activity in the laboratory is different to the number of haemocytes recorded in the field: the initial haemocyte number in the experiment reflects a hosts response to the initial stages of infection, whereas the number of haemocytes documented in infected samples in the field reflects host cellular immune activity once infection is established. In any case, both measures yield similar information regarding infection in the *Daphnia-Pasteuria* system: an increase in haemocyte number following parasite exposure predicts likely future infection (Auld et al. 2012a, Auld et al. 2012b, Auld et al. 2010).

The fifth *Daphnia* from each replicate was placed singly in a small jar with 60 ml of artificial medium, and medium was refreshed three times per week. The experiment was terminated on day 30, when all surviving hosts were placed in a 1.5 ml Eppendorf and stored at -20°C. Counts of *P. ramosa* transmission spores in each infected host were determined as follows: individual *Daphnia* were homogenized with 100 μl of ddH_2O, and two independent counts were made from the resulting suspension using a Neubauer (Improved) counting chamber (0.0025 $mm^2 \times 0.1$ mm depth).

Experiment 2

We ran a second experiment to better study parasite-induced mortality. Thus, following exposure to the parasites, infected hosts were maintained until they died. Replicates were maintained in the same manner as those used in experiment 1, and exposure protocols were also the same. There were two parasite treatments: hosts were either exposed to *P. ramosa* collected at the beginning of the season (July 8th) or at the end of the season (September 2nd). There were 100 replicates per parasite treatment, per genotype and thus a total of 400 replicates. Each replicate was exposed

222 individually to 5×10^4 spores for five hours. After the exposure period, replicates
223 were changed into new jars and fresh medium, and medium was then refreshed three
224 times per week. Jars were monitored daily for mortality, and infection status was
225 scored by eye.
226

227 **Analysis of field data**

228 All data were analysed using general linear models implemented in the R statistical
229 package (R 2005), and for all models, the significance of the predictor variables was
230 examined hierarchically using a stepwise backward model reduction procedure
231 (Crawley 2007).

232 First, we examined how ecological variables associated with the numbers of
233 parasite spores from infected hosts. This was done by testing the effects of
234 temperature, $\log_{10}$[infected host density] and $\log_{10}$[number of haemocytes] on the
235 $\log_{10}$-transformed parasite spore counts. Infected host density was used as a proxy for
236 the availability of susceptible hosts. All two-way interactions were also included as
237 explanatory variables and sample location nested within sample date was included as
238 a random effect. We tested the significance of the random effects by removing them
239 from the model and analysing the resulting change in deviance using a likelihood ratio
240 test.

241 Next, we analysed the number of haemocytes circulating in the host's
242 haemolymph, our measure of host immune activity. This was done by testing the
243 effects of host infection status (infected or not), pond temperature, parasite prevalence
244 and $\log_{10}$[host density] on $\log_{10}$-transformed haemocyte count data; all two-way
245 interactions again also included as explanatory variables and sample site nested within
246 sample date were included as random effects.

247

248 **Analysis of experimental data**

249 First we analysed parasite infection traits from experiment 1. We analysed the
250 probability of infection by fitting a generalized linear model with a binomial error
251 structure and a logit link function to the infection data (from parasite-exposed hosts
252 only); host genotype, parasite sample and their interaction were fitted as fixed factors.
253 Then, using data from infected hosts only, we analysed the number of parasite
254 transmission spores using a two-way ANOVA with the same model structure.

255 Next, we examined the host cellular response from experiment 1. This was
256 done using a two-way ANOVA, where host genotype, parasite exposure (exposed or
257 non-exposed control) and their interaction were fitted as fixed factors. Then, using
258 data from parasite-exposed hosts only, we tested whether parasite sample had an
259 effect on haemocyte counts, again using a two-way ANOVA, but with host genotype,
260 parasite sample and their interaction fitted as fixed factors. In all cases where the data
261 were non-orthogonal, type III sums of squares were used.

262 Second, we examined the host survival data from experiment 2. Specifically,
263 we tested whether host survival depended on whether they were exposed to *P. ramosa*
264 spores from the beginning or the end of the season. This was done using a Cox's
265 proportional hazards analysis applied to data from infected hosts only, where host
266 genotype and parasite sample fitted as fixed factors. All data used in these analyses
267 are archived at Dryad (DOI: xxx; to be determined upon acceptance).

268

RESULTS

Field data

Pasteuria-infected *Daphnia* were observed from early June until mid-September, during which pond temperatures varied between 12.1°C and 18.5°C. Parasite prevalence (the proportion of hosts that became infected) peaked twice: in early July and in mid August (Fig. 1A). The number of parasite transmission spores per infected host increased dramatically over the season ($\chi^2 = 6.56$, $p < 0.001$): infected hosts collected in late September had over ten-fold more spores than those collected in early June (Fig. 1B). Sample site within the pond did not explain a significant proportion of variation in the data ($\chi^2 = 7.11 \times 10^{-15}$, $p = 0.99$). After testing the effects of infected host density, infected host haemocyte number and pond temperature (and all two-way interactions) on the number of *P. ramosa* transmission spores, only infected host density remained significant after model reduction: *P. ramosa* transmission spore count was negatively associated with the density of infected hosts ($\log_{10}[y] = 5.76 - 0.49\log_{10}[x]$, $t_{12} = 3.49$, $p < 0.01$; Fig. 1C).

Finally, confirming previous work (Auld et al. 2012b), parasitized *Daphnia* had consistently more circulating haemocytes than their healthy counterparts ($F_{1,7} = 155.26$, $p < 0.0001$), but haemocyte number was not associated with pond temperature, parasite prevalence or host density. Further, haemocyte number did not vary over the course of the season ($\chi^2 = 0.19$, $p = 0.66$) or across sample sites ($\chi^2 = 6.40 \times 10^{-10}$, $p = 0.99$).

Experiment 1

Pasteuria ramosa collected at the end of the season was more infectious than *P.*

ramosa collected earlier (Table 1, Fig. 2), and the final number of *P. ramosa* transmission spores per infected host also depended on the host genotype and the parasite sample date (Table 2, Fig. 3). Further, *Daphnia* mounted a cellular response to *P. ramosa*: parasite-exposed hosts had 1282 ± 53 haemocytes, whereas unexposed controls had 604 ± 43 haemocytes (see Table 1). The strength of this cellular response also depended on the specific combination of host genotype and parasite sample date (Table 1), and the number of circulating haemocytes mirrored the proportion of infected hosts in a particular treatment (Fig. 2).

Experiment 2

Again, *P. ramosa* from the end of the season caused more infections than *P. ramosa* from the beginning of the season ($\chi^2 = 64.36$, $p < 0.0001$). Survival of infected hosts, the focus of this experiment, differed between the host genotypes: KA53 hosts survived 48.00 ± 1.25 days whereas KA62 hosts survived 54.38 ± 1.42 days ($\chi^2 = 9.81$, $p < 0.01$). The data also suggest *P. ramosa* collected at the end of the season kill their hosts later than *P. ramosa* collected at the beginning of the season (though this trend is not significant at the 0.05 level): hosts infected with spores collected on July 8th survived 50.20 ± 1.08 days, whereas hosts infected with spores collected on September 2nd survived 52.92 ± 2.29 days ($\chi^2 = 3.68$, $p = 0.06$).

Discussion

Our survey of a wild population revealed substantial fluctuation in the prevalence of the bacterium *P. ramosa*, including two peaks where 60-70% of their *Daphnia* hosts were infected. The number of *P. ramosa* transmission spores within infected hosts was 10 times greater in the autumn than in late spring and, concomitant with this

318 increase in spore numbers, the density of infected hosts declined dramatically.
319 Haemocyte counts were also higher in parasitized *Daphnia* than in their healthy
320 counterparts, consistent with an earlier study (Auld et al. 2012b).
321 These shifts in infection traits may be due to genetic changes in the parasite
322 population, but they may also be due to unmeasured environmental changes in the
323 pond or to demographic changes in the host population. For example, infected hosts
324 collected late in the season could simply be older hosts that have been infected for
325 longer and thus have allowed more time for *P. ramosa* proliferation. In this case, we
326 might not expect parasites collected late-season to differ from those collected earlier.
327 To shed light on this, we brought field-collected parasites into the laboratory
328 throughout the season and preserved them frozen until we could perform an
329 experiment. This experiment addressed whether parasites from different time points
330 expressed different trait values in a 'common garden', where the parasite samples
331 were exposed to a standard set of host genotypes and infection duration and dose was
332 the same for all parasitized hosts. The experiment showed that parasite infectivity,
333 capacity for immunostimulation and within-host growth depended on the date when
334 the parasite spores were initially collected from the wild (Table 1, Table 2).
335 These combined field and laboratory observations of phenotypic change in the
336 parasite population are compatible with the hypothesis of genetic change (i.e.,
337 evolution) in the parasite population. This could be in response to host evolution
338 (often observed in response to parasitism in *D. magna* populations, e.g., Duncan et al.
339 2007), or because genetically different parasite migrants (either from another
340 population or from the parasite 'seed bank' in the pond sediment) entered the
341 population. The genetic diversity of parasites within each infected host could also
342 play a role in the observed patterns. For example if parasite within-host diversity were

lower at the end of the season, this could alter within-host competition, with knock-on effects on virulence.

However, a set of explanations that do not rely upon genetic change in the parasite population cannot be excluded. For example, if hosts from the end of the growing season provided a higher quality environment for the parasites, the resulting transmission spores (which we collected and stored) could have been of higher quality, including superior transmissibility, yet genetically identical to early-season spores. A previous study of the *Daphnia-Pasteuria* system found that the amount of food consumed by hosts appears to affect parasite transmission spores and infection related traits on future hosts: well-fed hosts produced more virulent spores (i.e., spores that killed their hosts earlier: Little et al. 2008). Our findings are consistent with this as we found that parasites collected at the end of the season (when food quantity is presumably much lower) tended to be less virulent. However, that earlier study also found the quantity of food consumed by the host had no effect on the infectivity of parasite spores on future hosts, whereas we found a marked increase in parasite infectivity over time.

If natural selection has played a role in the rapid changes in parasite traits, it is intriguing that these traits were negatively correlated with infected host abundance (Fig. 1C). Theory on the relationship between density and virulence predicts that high availability of hosts should favour high parasite growth, virulence and transmission (Bull and Levin 1994, Day and Gandon 2007, Ewald 1994), and our observations clearly do not fit this. This lack of fit is perhaps not surprising since *P. ramosa* infects from its diapausing stage, and standard theory considers directly horizontally transmitted parasites. Parasites with long-lived externally viable stages are predicted to cause high virulence irrespective of transmission opportunities because the relative

cost of virulence will be low, assuming the costs of waiting to infect are also low (i.e., when there is little degradation in spore infectivity over time: (Bonhoeffer et al. 1996, Ewald 1994, Walther and Ewald 2004). Given the robust ability of *P. ramosa* to 'wait' for hosts (Decaestecker et al. 2007), we would expect them to evolve consistently high virulence. Thus, theory on 'sit and wait' strategies also does not explain the observed change in parasite growth rate across a season. Clearly, *P. ramosa*'s capacity for diapause, both in terms of natural selection on virulence, and for 'migration' from the seed bank, and will need to be considered further.

Improving our understanding how parasites change over the course of epidemics in wild host populations is important for both pure and applied questions in evolutionary biology. Our findings point towards the evolution of increased parasite infectivity. However, parasite within-host growth, virulence and capacity for immunostimulation, amongst other factors we have not speculated upon here, could have changed as a result of both genetic and non-genetic change in the parasite population. Our findings thus highlight the need to go back into the wild and increase the understanding the natural history of host-parasite systems.

Acknowledgements

S.K.J.R.A was funded by a NERC studentship and T.J.L. and P.J.W were funded by a Wellcome Trust Senior Research Fellowship in Basic Biomedical Sciences to T.J.L.

References

Auld, S. K. J. R., Edel, K. H. and Little, T. J. 2012a. The cellular immune response of *Daphnia magna* under host-parasite genetic variation and variation in initial dose. - Evolution 66: 3287-3293.

393 Auld, S. K. J. R., Graham, A. L., Wilson, P. J. and Little, T. J. 2012b. Elevated
394 haemocyte number is associated with infection and low fitness potential in wild
395 *Daphnia magna*. - Functional Ecology 26: 434-440.
396 Auld, S. K. J. R., Hall, S. R. and Duffy, M. A. 2012c. Epidemiology of a *Daphnia*-
397 multiparasite system and its implications for the Red Queen. - PLOS ONE 7: e39564.
398 Auld, S. K. J. R., Scholefield, J. A. and Little, T. J. 2010. Genetic variation in the
399 cellular response of *Daphnia magna* (Crustacea: Cladocera) to its bacterial parasite. -
400 Proceedings of the Royal Society of London Series-B Biological Sciences 277: 3291-
401 3297.
402 Bangham, C., Anderson, R., Baquero, F., Bax, R., Hastings, I., Koella, J., McLean,
403 A., Smith, T., Taddei, F. and Levin, B. 1999. Evolution of infectious diseases: the
404 impact of vaccines, drugs, and social factors. - In: Stearns, S. C. (ed.) Evolution in
405 Health and Disease. Oxford University Press.
406 Blanford, S., Thomas, M. B., Pugh, C. and Pell, J. K. 2003. Temperature checks the
407 Red Queen? Resistance and virulence in a fluctuating environment. - Ecology Letters
408 6: 2-5.
409 Bonhoeffer, S., Lenski, R. E. and Ebert, D. 1996. The curse of the pharaoh: The
410 evolution of virulence in pathogens with long living propagules. - Proceedings of the
411 Royal Society of London Series-B Biological Sciences 263: 715-721.
412 Bull, J. J. and Levin, B. R. 1994. Evolution of Infectious Diseases. - Science 265:
413 1469-1470.
414 Crawley, M. J. 2007. The R book. - John Wiley & Sons, Ltd.
415 Day, T. and Gandon, S. 2007. Applying population-genetic models in theoretical
416 evolutionary epidemiology. - Ecology Letters 10: 876-888.
417 Decaestecker, E., Gaba, S., Raeymaekers, J. A. M., Stoks, R., Van Kerckoven, L.,

418 Ebert, D. and De Meester, L. 2007. Host-parasite 'Red Queen' dynamics archived in
419 pond sediment. - Nature 450: 870-873.
420 Duffy, M. A., Cáceres, C. E., Hall, S. R., Tessier, A. J. and Ives, A. R. 2010.
421 Temporal, spatial and between-host comparisons of patterns of parasitism in lake
422 zooplankton. - Ecology 91: 3322-3331.
423 Duneau, D., Luijckx, P., Ben-Ami, F., Laforsch, C. and Ebert, D. 2011. Resolving the
424 infection process reveals striking differences in the contribution of environment,
425 genetics and phylogeny to host-parasite interactions. - BMC Biology 9: 11.
426 Ebert, D. 2005. Ecology, epidemiology and evolution of parasitism in *Daphnia*. -
427 National Library of Medicine (US), National Center for Biotechnology Information.
428 Ebert, D. 2008. Host-parasite coevolution: Insights from the *Daphnia*-parasite model
429 system. - Current Opinion in Microbiology 11: 290-301.
430 Ebert, D., Rainey, P., Embley, T. M. and Scholz, D. 1996. Development, life cycle,
431 ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888:
432 Rediscovery of an obligate endoparasite of *Daphnia magna* Straus. - Philosophical
433 Transactions of the Royal Society of London Series B-Biological Sciences 351: 1689-
434 1701.
435 Ewald, P. W. 1994. The Evolution of Infectious Disease. - Oxford University Press.
436 Hall, S. R., Knight, C. J., Becker, C. R., Duffy, M. A., Tessier, A. J. and Cáceres, C.
437 E. 2009. Quality matters: resource quality for hosts and the timing of epidemics. -
438 Ecology Letters 12: 118-128.
439 Jensen, K. H., Little, T. L., Skorping, A. and Ebert, D. 2006. Empirical support for
440 optimal virulence in a castrating parasite. - PLOS Biology 4: 1265-1269.
441 King, K. C., Auld, S. K. J. R., Wilson, P. J., James, J. and Little, T. J. 2013. Bacterial
442 parasites not killed by resistant *Daphnia*: implications for host-mediated selection. -

443 Ecology and Evolution 3: 197-203.
444 Kluttgen, B. U., Dulmer, U., Engels, M. and Ratte, H. T. 1994. ADaM, an artificial
445 freshwater for the culture of zooplankton. - Water Research 28: 743-746.
446 Krist, A. C., Jokela, J., Wiehn, J. and Lively, C. M. 2004. Effects of host condition on
447 susceptibility to infection, parasite development rate, and parasite transmission in a
448 snail-trematode system. - Journal of Evolutionary Biology 17: 33-40.
449 Lavine, M. D., Chen, G. and Strand, M. R. 2005. Immune challenge differentially
450 affects transcript abundance of three antimicrobial peptides in hemocytes from the
451 moth *Pseudoplusia includens*. - Insect Biochemistry and Molecular Biology 35: 1335-
452 1346.
453 Luijckx, P., Fienberg, H. and Duneau, D. 2011. Resistance to a bacterial parasite in
454 the crustacean *Daphnia magna* shows Mendelian segregation with dominance. -
455 Heredity.
456 Marchese, A., Schito, G. C. and Debbia, E. A. 2000. Evolution of antibiotic resistance
457 in gram-positive pathogens. - Journal of Chemotherapy 12: 459-462.
458 Paterson, S. and Barber, R. 2007. Experimental evolution of parasite life-history traits
459 in *Strongyloides ratti* (Nematoda). - Proceedings of the Royal Society of London
460 Series-B Biological Sciences 274: 1467-1474.
461 R. 2005. R: A Language and Environment for Statistical Computing. - R Foundation
462 for Statistical Computing.
463 Vale, P. F., Best, A., Boots, M. and Little, T. J. 2011. Epidemiological, evolutionary
464 and coevolutionary implications of context-dependent parasitism. - American
465 Naturalist 177: 510-521.
466 Walther, B. A. and Ewald, P. W. 2004. Pathogen survival in the external environment
467 and the evolution of virulence. - Biological Reviews of the Cambridge Philosophical

Society 79: 849-69.

FIGURE LEGENDS

Figure 1. (A) *Daphnia* population density (mean ±1 S.E., bold circles) and mean *P. ramosa* prevalence (grey dashed line) over time; (B) the number of *P. ramosa* transmission spores per infected host (mean ±1 S.E.) over time; and (C) the association between the numbers of *P. ramosa* transmission spores per infected host and the density of infected infected hosts (both mean ±1 S.E.).

Figure 2. The proportion of infected *Daphnia* (bars, right axis) and the number of haemocytes per microlitre (mean ± 1 S.E., circles) in four host genotypes exposed to parasite samples from a wild population. Zeroes indicate treatments where no infections occurred.

Figure 3. Number of *P. ramosa* transmission spores per infected host (mean ± 1 S.E.) in four host genotypes exposed to parasite samples from a wild population.

Table 1. The effects of host genotype and parasite sample date on the probability of infection and host cellular immune response.

	DF	LR-χ^2	p
Probability of infection (parasite-exposed hosts)			
Host genotype	3	0.70	0.87
Parasite sample	6	133.83	< 0.0001
Post genotype x Parasite sample	18	16.75	0.54
Error	278		
	DF	F	p
Log-$_{10}$[haemocytes] (all hosts)			
Host genotype	3	3.57	< 0.05
Parasite exposure	1	27.02	< 0.0001
Host genotype x Parasite exposure	3	2.11	0.10
Error	328		
	DF	F	p
Log-$_{10}$[haemocytes] (parasite-exposed hosts)			
Host genotype	3	6.16	< 0.001
Parasite sample	5	22.80	< 0.0001
Host genotype x Parasite sample	15	2.03	< 0.05
Error	263		

509 **Table 2.** The effects of host genotype and parasite sample date on the

510 number of parasite transmission spores in infected hosts.

	DF	F	p
Log_{10}[transmission spores] (infected hosts)			
Host genotype	3	14.28	< 0.0001
Parasite sample	4	7.20	< 0.0001
Host genotype x Parasite sample	10	1.01	0.44
Error	85		

511
512
513
514
515
516
517