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Parasite transmission in a natural multihost–multiparasite community

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Understanding the transmission and dynamics of infectious diseases in natural communities requires understanding the extent to which the ecology, evolution and epidemiology of those diseases are shaped by alternative hosts. We performed laboratory experiments to test how parasite spillover affected traits associated with transmission in two co-occurring parasites: the bacterium *Pasteuria ramosa* and the fungus *Metschnikowia bicuspidata*. Both parasites were capable of transmission from the reservoir host (*Daphnia dentifera*) to the spillover host (*Ceriodaphnia dubia*), but this occurred at a much higher rate for the fungus than the bacterium. We quantified transmission potential by combining information on parasite transmission and growth rate, and used this to compare parasite fitness in the two host species. For both parasites, transmission potential was lower in the spillover host. For the bacterium, virulence was higher in the spillover host. Transmission back to the original host was high for both parasites, with spillover influencing transmission rate of the fungus but not the bacterium. Thus, while inferior, the spillover host is not a dead-end for either parasite. Overall, our results demonstrate that the presence of multiple hosts in a community can have important consequences for disease transmission, and host and parasite fitness.

This article is part of the themed issue 'Opening the black box: re-examining the ecology and evolution of parasite transmission'.

1. Introduction

Infectious diseases are a threat to almost all living organisms. As a result, there is widespread interest in understanding the factors influencing the epidemiology, ecology and evolution of host–parasite systems. One factor that is likely to be important is that, in nature, parasites commonly encounter multiple potential host species that vary in both quantity and quality, leading to heterogeneous and asymmetric transmission among and between host species [1–4]. Differences in susceptibility of hosts in a community can have important impacts on disease dynamics, including driving patterns of spillover and dilution. Spillover occurs when sufficiently large epidemics in susceptible (reservoir) hosts cause otherwise resistant host species to suffer infections as a result of elevated exposure to parasite transmission stages [2,5]. Conversely, parasites that infect a host species that poorly transmits to subsequent hosts can drive a decline in parasite transmission stages in the environment, and potentially reduce disease prevalence in other more susceptible host species. This is termed the dilution effect [6].

Theory predicts that parasites should evolve greater transmission rates in higher quality hosts, potentially at a cost to the ability to transmit to lower quality, diluting hosts [7]. However, if the relative quality and/or quantity of different host species fluctuate, or if the higher quality host is relatively rare, we might see the evolution of a more generalist strategy across hosts, because a specialist strategy will more likely result in extinction (e.g. [8]). In addition to influencing infectivity, community context will also play an important role in shaping the virulence of each parasite species. On the one hand, multihost parasites may evolve higher virulence on their high quality hosts [7]; on the

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other hand, they may evolve runaway virulence on their rarer (low quality) hosts and optimal virulence on their main (high quality) hosts if spillover is rare [1,7]. To complicate matters, individual hosts commonly encounter multiple potential parasites over their lifetime, so interactions with one parasite will likely influence ecological and evolutionary interactions with other parasite species. As multihost–multiparasite communities are the norm and not the exception, the ecology and evolution of infectious diseases are dependent on the various hosts and parasites in a natural community [1,3,7,9]. However, most studies of host–parasite interactions have overlooked this complexity [3,10,11]. Thus, a major outstanding challenge is to quantify how spillover and dilution affect patterns of disease transmission and virulence in multihost–multiparasite communities.

We conducted controlled laboratory experiments to examine the effects of spillover on traits associated with parasite transmission in a natural multihost–multiparasite community. The hosts were the freshwater crustaceans *Daphnia dentifera* (the reservoir host, where infections are common) and *Ceriodaphnia dubia* (where infections are comparatively rare) and the parasites were the sterilizing bacterial parasite *Pasteuria ramosa* and the lifespan-reducing fungal parasite *Metschnikowia bicuspidata*. All hosts and parasites co-occur in the same population. We found that interspecific transmission rates, within-host growth and virulence differed between the bacterial and fungal parasites. In addition, passage of the fungal parasite through the spillover host increased parasite transmission rate when re-exposed to the focal host. Passage of the bacterium through the spillover host did not affect transmission back to the reservoir host. In summary, we show that two parasites with similar infection mechanisms exhibit different patterns of transmission and virulence across reservoir and spillover hosts.

2. Material and methods

(a) Hosts and parasites

Ceriodaphnia dubia and *Daphnia dentifera* (hereafter: *Ceriodaphnia* and *Daphnia*, respectively) are both common freshwater zooplankton found in stratified lakes in Midwestern North America [12]. They are cyclically parthenogenetic, which allows the maintenance of clonal, isofemale lines in the laboratory. Both species suffer infections with the bacterium, *P. ramosa*, and the fungus, *M. bicuspidata* [13,14], though co-infections are rare (MA Duffy 2013-16, unpublished data). Spores of either parasite are consumed alongside food during host filter-feeding [15,16], cross the gut wall and undergo replication within the haemocoel; mature transmission spores are then released upon host death [17,18]. However, while both parasites are horizontally transmitted obligate killers, they have different effects on host fitness in *Daphnia* spp.: *P. ramosa* (hereafter: bacterium) causes host sterilization but has a limited effect on host lifespan [14,19], whereas *M. bicuspidata* (hereafter: fungus) kills its host early, but does not strongly limit fecundity prior to death [14,20,21].

Healthy *Ceriodaphnia* and *Daphnia*, and both *Pasteuria*- and *Metschnikowia*-infected *Daphnia* were collected from Dogwood Lake, Sullivan County, Indiana, USA during 2011. Eight *Ceriodaphnia* isofemale lines (named C1, C2, C5, C7, C22, C23, C27 and C30) and 10 *Daphnia* isofemale lines (named D1, D3, D4, D6, D7, D13, D14, D23, D25 and D26) were maintained clonally in the laboratory. Parasite cultures were established as follows: five *Pasteuria*-infected and seven *Metschnikowia*-infected *Daphnia* were homogenized and pooled according to parasite species; the spore cultures were each propagated by exposing four *Daphnia* genotypes (D1, D4, D14 and D26) to them for three rounds of infection for *Pasteuria* and 5–7 rounds of infection for *Metschnikowia*.

(b) Experiment 1: magnitude of spillover for *Pasteuria* and *Metschnikowia* parasites

The aim of this experiment was to quantify the magnitude of spillover and the consequences for virulence of both parasites. Fifteen to 25 replicate lines were established for each host isofemale line (henceforth 'line') of *Ceriodaphnia* and *Daphnia*. Replicates consisted of two neonates kept in 40 ml of media (50% artificial *Daphnia* medium [22] and 50% filtered lake water), and were maintained under standard conditions: 20°C, 16 : 8 light/dark cycle and fed 1×10^6 *Ankistrodesmus falcatus* algal cells per animal per day. Maternal lines were maintained for three generations to minimize variation due to maternal effects. Once they had reached the third generation, a single neonate from the second clutch of each maternal replicate was allocated to one of two treatments: parasite-exposed or control.

Experiment 1 was blocked according to parasite (block 1: bacterium, block 2: fungus). Replicates consisted of a single animal in 40 ml of media. In each block, there were 12–19 parasite-exposed replicates and 4–8 control replicates per line (some replicates died during the parasite exposure period and were excluded). Bacteria-exposed animals received 2000 spores ml^{-1}, fungus-exposed animals received 500 spores ml^{-1} and controls received a 100 µl aliquot of crushed healthy *Daphnia*; doses were selected to achieve comparable prevalence of infection for each parasite in the reservoir (*Daphnia*) host (see [14]). Treatment exposure lasted 48 h, during which replicate animals were fed 0.5×10^6 algal cells per animal. After treatment exposure, all animals were transferred into clean beakers with fresh media. Beakers were checked daily for host mortality and offspring production (offspring were counted and discarded), and fed the standard food amount. Media was changed three times per week. On the day of death, each animal was placed individually in 1.5 ml microcentrifuge tubes, homogenized in 100 µl ddH_2O, and the densities of mature spores were determined using a haemocytometer (see [18] for protocols).

Data from the bacteria and fungus experimental blocks were analysed separately using R. (Data and code are deposited at Dryad: doi:10.5061/dryad.3jm7h) We analysed infection risk (proportion of infected hosts) by fitting generalized linear mixed models (GLMM) with binomial errors to data from parasite-exposed hosts (i.e. excluding controls); host species was fitted as a fixed factor and host individual within line within host species was fitted as a nested random effect. Parasite burden in infected hosts was also analysed using a GLMM fitted to spore counts from infected hosts; the random effects structure was the same as the previous model. For both analyses, we determined the significance of host line within species by comparing models with the full random effect with models where only host individual was fitted as a random effect using likelihood ratio test. Finally, we calculated a metric for the overall transmission potential of each parasite for each *Ceriodaphnia* and *Daphnia* line. The overall transmission potential is the product of the parasite transmission rate (β) and the parasite growth rate, i.e. the density of spores divided by host lifespan (σ/τ). Values of β were determined for each host line and parasite using the following equation:

$$p = \frac{1 - S_t}{S_0} = 1 - \exp(-\beta Z_0 t),$$

where p is the proportion of hosts infected for a particular line, S_t is the density of uninfected hosts at the end of exposure

Table 1. Mean density of spores from first host (from experiment 1), number of infected first hosts, scaled total spores (spore density assuming equal numbers of infections for spillover and reservoir species) and the doses given to experiment 2 replicates.

	spores per individual, σ_1 (first host)	number infected first hosts	scaled total spores	exp. 2 spore dose (ml^{-1})
(a) bacterium				
Ceriodaphnia	248 333	4	794 667	1324
Daphnia	963 522	54	3 083 270	5139
ref strain	—	—	—	2000
(b) fungus				
Ceriodaphnia	13 208	25	264 167	440
Daphnia	50 545	54	1 108 970	1685
ref strain	—	—	—	500

Table 2. Summary of analyses of experiment 1 data on the proportion of infected hosts following parasite exposure (infectivity), parasite growth measured at host death, host survival and host fecundity.

	infectivity	parasite density (infected only)	host survival	host fecundity (exposed only)
(a) bacterium				
infection	—	—	$\chi^2_1 = 17.72^{***}$	$\chi^2_1 = 39.57^{***}$
host species	$\chi^2_1 = 7.00^{**}$	$\chi^2_1 = 1.78$	$\chi^2_1 = 0.78$	$\chi^2_1 = 4.75^{*}$
infection × host spp.	—	—	$\chi^2_1 = 13.01^{***}$	$\chi^2_1 = 0.84$
host line (host spp.)	$\chi^2_1 = 19.26^{***}$	$\chi^2_1 = 11.70^{***}$	—	—
(b) fungus				
infection	—	—	$\chi^2_1 = 279.63^{***}$	$\chi^2_1 = 227.94^{***}$
host species	$\chi^2_1 = 4.97^{*}$	$\chi^2_1 = 7.76^{**}$	$\chi^2_1 = 3.10$	$\chi^2_1 = 7.67^{**}$
infection × host spp.	—	—	$\chi^2_1 = 1.92$	$\chi^2_1 = 1.94$
host line (host spp.)	$\chi^2_1 = 2.35$	$\chi^2_1 = 0.69$	—	—

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

time t, S_0 is the initial density of hosts, Z_0 is the density of parasite spores to which the hosts were exposed and t is the duration of exposure in days. These genotypic values for β were multiplied by (σ/τ) values for each infected host. We tested for an effect of spillover on overall transmission potential for each parasite by comparing $\beta(\sigma/\tau)$ (that is, transmission potential) between *Ceriodaphnia* and *Daphnia* using Welch's t-tests.

We then examined the fitness consequences of infection in terms of host survival (for parasite-exposed hosts only), host fecundity and parasite growth. Host survival was analysed using mixed effects Cox's Proportional Hazards analysis (*coxme* package) models with infection status (infected or not), host species and the interaction fitted as fixed effects; individual within line within host species was fitted as a nested random effect. We analysed host fecundity by fitting a GLMM with quasi-Poisson errors (to account for overdispersion) to offspring count data from parasite-exposed hosts; infection status and host species were fitted as fixed factors and individual within line within host species was fitted as a nested random effect.

Next, we examined how the relationship between square root-transformed parasite growth rate (parasite burden/age of host at death) and square root-transformed host reproductive rate (total host fecundity/age of host at death) was mediated by the identity of the host; this was done using a linear mixed effects model (LME), where reproductive rate and host species were fitted as fixed factors and host line was fitted as a random effect. We did this for fungus-infected hosts only; the lack of bacterium-infected *Ceriodaphnia* prevented us from testing the effect of host species. Finally, we tested the extent to which the relationship between parasite burden and host day of death was dependent on host species. This was also done using a LME with the same random effects structures.

(c) Experiment 2: how does spillover affect transmission to the original *Daphnia* host?

This experiment was designed to quantify the magnitude of transmission back to the original reservoir host from the spillover host. Parasite spores from infected animals in experiment 1 were used alongside reference isolates. Methods for experiment 2 were similar to those of experiment 1. Twelve replicate maternal lines of three *Daphnia* lines were established (lines D1, D3, D7). Each replicate consisted of six neonate *Daphnia* kept in 100 ml media. Replicates were maintained under standard conditions (see above) for three generations.

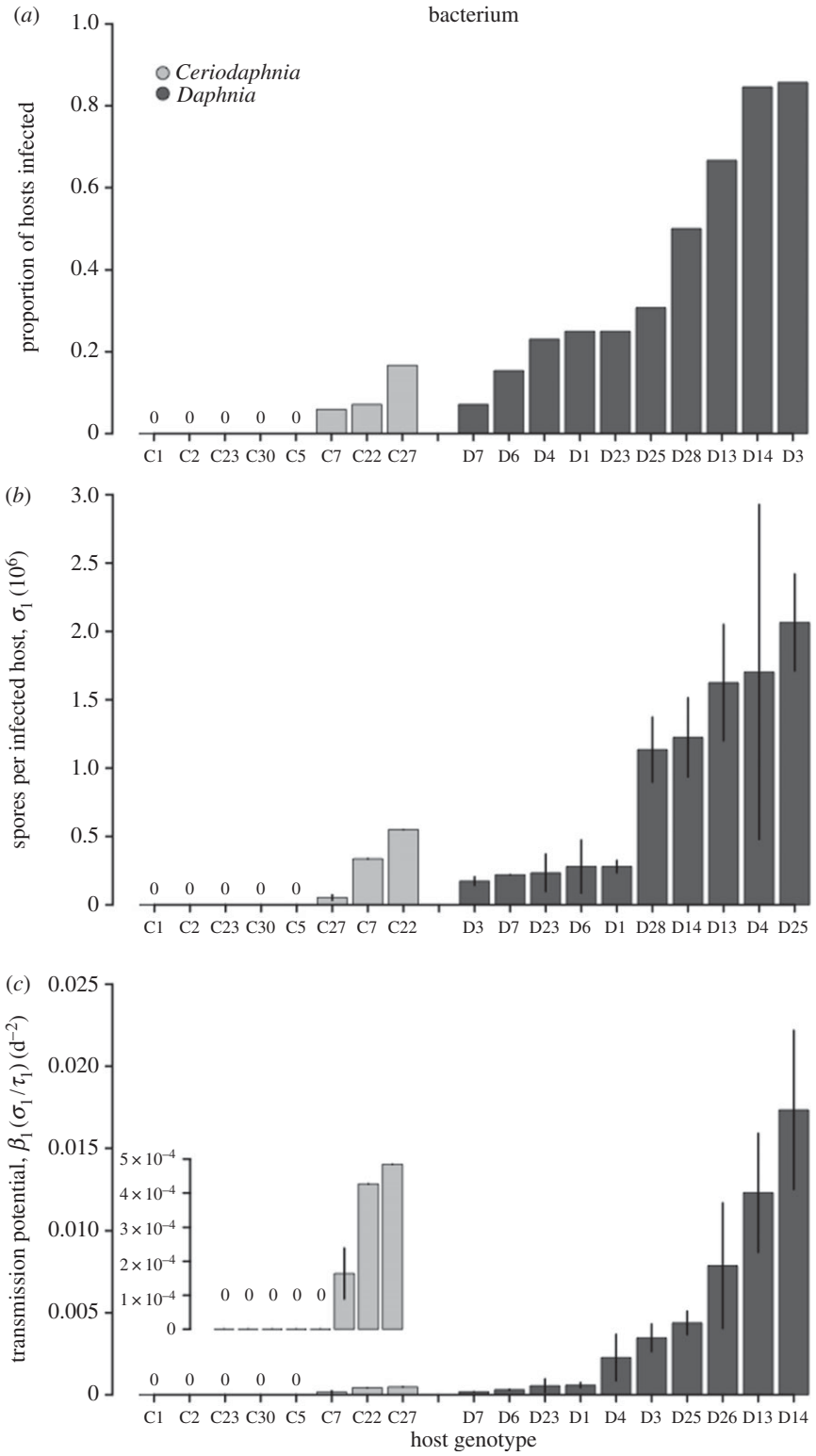

Figure 1. (*a*) Infectivity, (*b*) within-host growth and (*c*) overall transmission potential of the bacterium *P. ramosa* in its reservoir host, *D. dentifera* and spillover host, *C. dubia*. Note that the placement of a particular genotype can shift between panels.

Infected samples from experiment 1 were thoroughly mixed with a pipette. Eighty microlitres of each sample was grouped according to the species of its host. This approach was taken to yield sufficient spore doses. Spore samples varied in volume (between 0.32 and 4.32 ml) depending on the number of infected animals per host species in experiment 1 (between 4 and 54). In nature, transmission to the second host will depend on: (i) the per-spore infectivity and (ii) the number of spores to which each host is exposed. For this part of the experiment, we controlled β to make it as though there had been equal numbers of infected *Ceriodaphnia* and *Daphnia* in the first experiment (table 1). This approach had two advantages: it allowed us to reasonably control for variation in initial parasite dose that results from variable parasite growth rates in the initial host; it also allowed us to simultaneously assess the effects of variation in per spore infectivity and parasite growth in the first host without the confounding effect of different numbers of host individuals of the two species. In summary, our experiment provides a scenario where equal numbers of reservoir and spillover hosts became infected and the spore production from those hosts was allowed to vary, but the metric of transmission (β) incorporates variation in parasite dose in such a way as to make it comparable across host species.

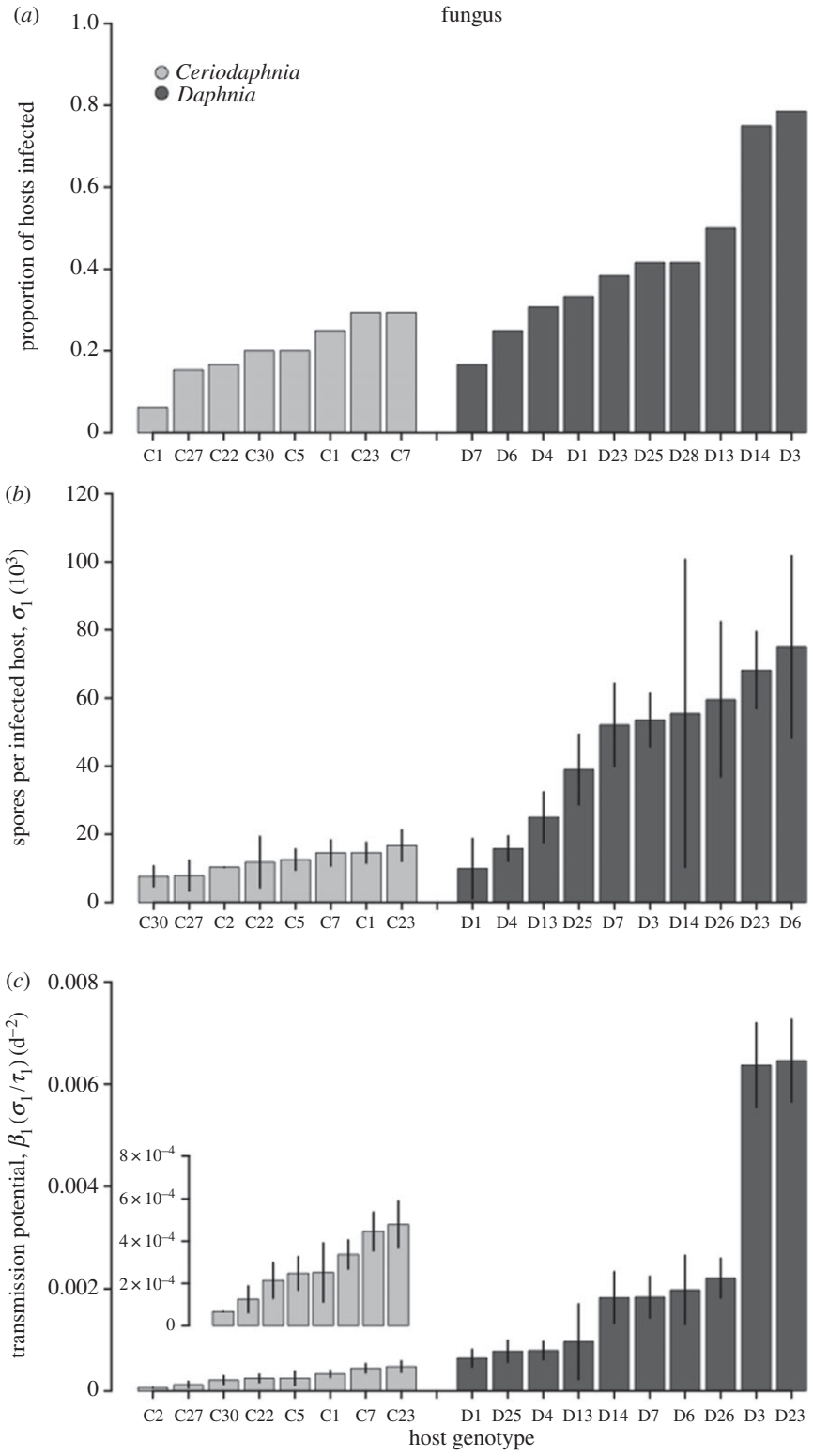

Figure 2. (*a*) Infectivity, (*b*) within-host growth and (*c*) overall transmission potential of the fungus *M. bicuspidata* in its reservoir host, *D. dentifera* and spillover host, *C. dubia*. Note that the placement of a particular genotype can shift between panels.

Replicates consisted of six *Daphnia* taken from the second clutch of the third maternal generation, and were maintained under standard conditions. *Daphnia* were transferred from 100 ml beakers to 50 ml beakers and were exposed to either 100 µl of one of the parasite samples from infections in the first experiment (see table 1 for spore doses for each sample) or to 100 µl of the reference parasite isolate used to infect animals in the first experiment (2000 spores ml^{-1} for *Pasteuria* and 500 spores ml^{-1} for *Metschnikowia*). There were four replicate beakers, six parasite treatments and three *Daphnia* lines, giving a total of 72 replicates. Treatment exposure lasted 48 h, during which replicate animals were fed 0.5×10^6 algal cells per animal (that is, half of the standard food amount). Following parasite exposure, all animals were transferred into clean 100 ml beakers with fresh media. Beakers were checked daily for host mortality and fed the standard food amount. Media was changed three times per week (and any offspring were removed). On the day of death, each animal was placed in a 1.5 ml microcentrifuge tube, homogenized in 100 µl ddH_2O, and the densities of mature spores was determined using a haemocytometer.

The data for the two parasites were again analysed separately using R. First, we examined how spillover influenced parasite

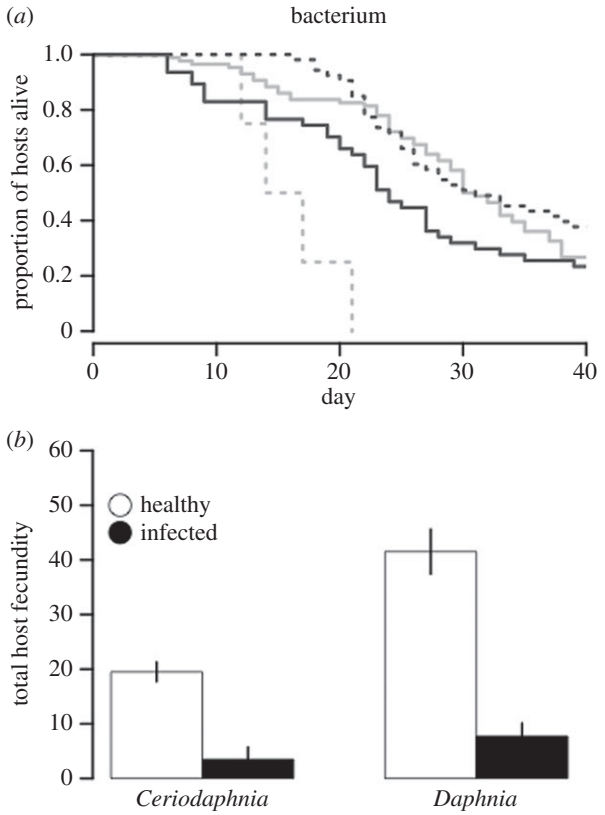

Figure 3. (*a*) Host survival in *D. dentifera* (dark grey lines) and *C. dubia* (light grey lines) that are either healthy (solid lines) or infected with the bacterium, *P. ramosa* (dashed lines); (*b*) host fecundity in healthy and *Pasteuria*-infected *Ceriodaphnia* and *Daphnia*.

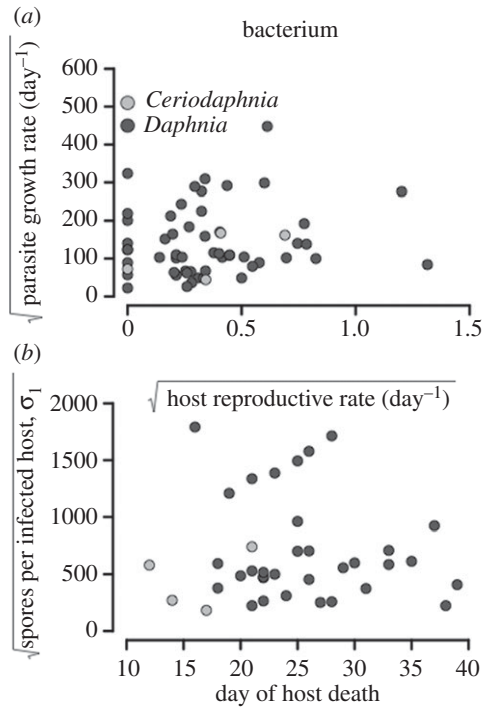

Figure 4. (*a*) Relationship between bacterial growth rate and host reproductive rate, and (*b*) relationship between parasite densities and host day of death for both the spillover host, *Ceriodaphnia*, or the reservoir host, *Daphnia*.

transmission to the original *Daphnia* host. We calculated parasite transmission rate (β) for each replicate beaker using the equation given above. For each parasite, we fitted a LME model (*nlme* package) to the β data, with the identity of the first host species fitted as a fixed factor and the identity of the second host (*Daphnia*) line fitted as a random effect. Next, we analysed both parasite growth rate (σ_1/τ_1) within infected hosts and overall transmission potential ($\beta(\sigma/\tau)$) using LMEs with the same model structure.

3. Results

(a) Greater spillover in the fungal parasite than in the bacterial parasite

The bacterium, *Pasteuria*, was much more infectious to *Daphnia* (mean: 41% infected) than to *Ceriodaphnia* (mean: 4% infected; table 2a; figure 1*a*). There was also considerable variation in bacterial infectivity within host species: the proportion of hosts infected depended on host line nested within host species (table 2a; figure 1*a*). Parasite densities at host death were significantly higher in *Daphnia* (mean: $9.64 \times 10^5 \pm 1.47 \times 10^5$) than in *Ceriodaphnia* (mean: $2.48 \times 10^5 \pm 1.21 \times 10^5$; table 2a) and also depended on host line nested within host species (table 2a; figure 1*b*). (Note that, throughout the results, the error values given are ± 1 standard error of the mean.) When we analysed the bacterial transmission potential ($\beta_1(\sigma_1/\tau_1)$) for each host line, we found it to be significantly higher in *Daphnia* ($4.93 \times 10^{-3} \pm 1.85 \times 10^{-3}$) than in *Ceriodaphnia* ($0.13 \times 10^{-3} \pm 0.07 \times 10^{-3}$; Welch's $t = 2.59$, DF $= 9.03$, $p = 0.029$; figure 1*c*).

The fungus was also more infectious to *Daphnia* (mean: 42% infected) than *Ceriodaphnia* (mean: 20% infected; table 2c). There was no significant variation in infectivity within host species (table 2b; figure 2*a*). Fungal within-host growth was significantly higher in *Daphnia* (mean: $5.05 \times 10^4 \pm 0.46 \times 10^4$) than in *Ceriodaphnia* (mean: $1.32 \times 10^4 \pm 0.15 \times 10^4$; table 2b; figure 2*b*), but did not depend on host line nested within host species (table 2b; figure 2*b*). Overall fungus transmission potential ($\beta_1(\sigma_1/\tau_1)$) was significantly higher in *Daphnia* ($2.38 \times 10^{-3} \pm 6.95 \times 10^{-4}$) than in *Ceriodaphnia* ($0.27 \times 10^{-3} \pm 0.51 \times 10^{-4}$; Welch's $t = 3.04$, DF $= 9.10$, $p = 0.014$; figure 2*c*).

(b) Effects of spillover on virulence differed between the two parasites

Bacterial infection reduced host survival in *Ceriodaphnia* but caused a small increase in survival in *Daphnia* (as evidenced by an infection status × host species interaction: table 2a; figure 3*a*). Bacterial infection caused an equally severe fecundity reduction in both host species (i.e. there was no infection × host species interaction: table 2a; figure 3*b*). In bacteria-infected *Daphnia*, there was no relationship between parasite growth rate (parasite density/host day of death) and host reproductive rate (host fecundity/host day of death, LME: $F_{1,43} = 2.69$, $p = 0.11$; see figure 4*a*), nor was there a relationship between bacterial spore burden and day of host death (LME: $F_{1,43} = 2.06$, $p = 0.16$; figure 4*b*). There were too few infected *Ceriodaphnia* for adequate analysis of these relationships.

Fungal infection caused similarly large reductions in survival for both host species (there was no infection status × host species interaction: table 2b; figure 5*a*). Fungal infection also caused equally severe reductions in host fecundity in both host species (table 2b and figure 5*b*). There was a

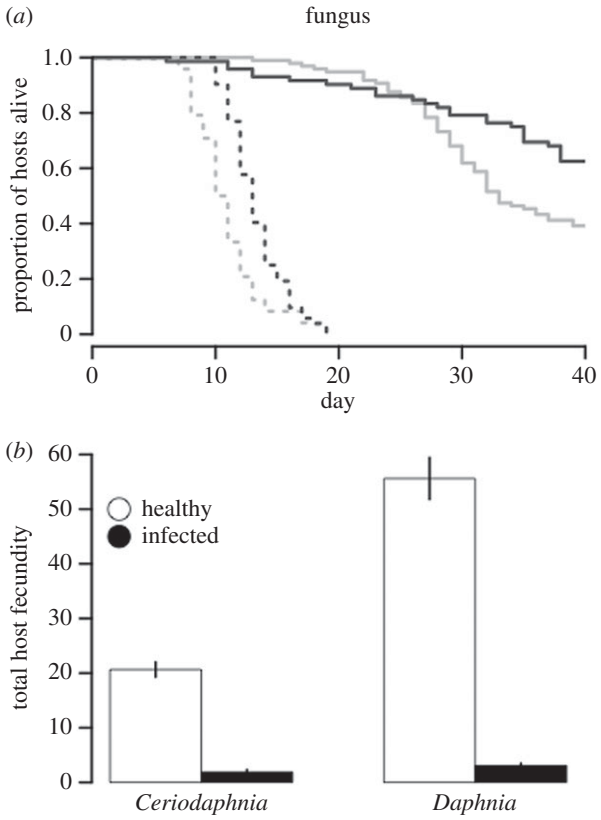

Figure 5. (*a*) Host survival in *D. dentifera* (dark grey lines) and *C. dubia* (light grey lines) that are either healthy (solid lines) or infected with the fungus, *M. bicuspidata* (dashed lines); (*b*) host fecundity in healthy and *Metschnikowia*-infected *Ceriodaphnia* and *Daphnia*.

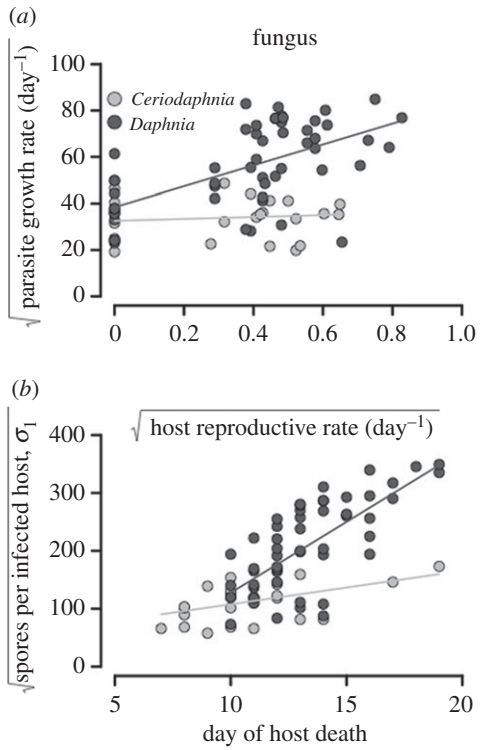

Figure 6. (*a*) Relationship between fungal growth rate and host reproductive rate, and (*b*) relationship between parasite densities and host day of death for both the spillover host, *Ceriodaphnia* or the reservoir host, *Daphnia*.

positive relationship between fungal growth rate and host reproductive rate in *Metschnikowia*-infected *Daphnia;* infected *Ceriodaphnia* did not show this positive relationship (i.e. there was a host reproductive rate × host species interaction, LME: $F_{1,56} = 9.19$, $p = 0.0037$; figure 6*a*). Finally, there was a positive relationship between fungal spore burden and day of host death (LME: $F_{1,56} = 16.44$, $p < 0.0002$), which was stronger for infected *Daphnia* than for infected *Ceriodaphnia* (day of host death × host species interaction: $F_{1,56} = 25.66$, $p < 0.0001$; figure 6*b*).

(c) Spillover influences patterns of fungal, but not bacterial, transmission to the original *Daphnia* host

For both parasites, passage through the spillover host, *Ceriodaphnia*, resulted in significantly fewer transmission spores than passage through the focal host, *Daphnia* (figures 1 and 2 and table 1a). In experiment 2, we examined how passage through either *Ceriodaphnia* or *Daphnia* affected parasite transmission rate (β_2) and overall transmission potential $\beta_2(\sigma_2/\tau_2)$ in the original (*Daphnia*) host species. For the bacterium, host species did not affect β_2 (LME: $F_{2,31} = 1.65$, $p = 0.209$), though there was some (marginally non-significant) evidence that passage through *Ceriodaphnia* could lead to reduced parasite growth rates (σ_2/τ_2) (LME: $F_{2,31} = 2.59$, $p = 0.091$). There was no effect of spillover on overall transmission potential $\beta_2(\sigma_2/\tau_2)$ (LME: $F_{2,31} = 1.31$, $p = 0.284$; figure 7).

For the fungus, passage through *Daphnia* resulted in lower β_2 than passage through *Ceriodaphnia* (LME: $F_{2,31} = 8.99$, $p = 0.0008$). There was no effect of host species on parasite growth rate (σ_2/τ_2) (LME: $F_{2,31} = 0.15$, $p = 0.863$). Overall fungal transmission potential, $\beta_2(\sigma_2/\tau_2)$, showed a similar pattern as β_2: passage through the spillover host (as opposed to the reservoir host) led to a marginally non-significant increase in overall transmission potential (LME: $F_{2,31} = 3.16$, $p = 0.052$; figure 8).

4. Discussion

Much of our understanding of the ecology and evolution of infectious disease comes from detailed examination of single host–single parasite systems. However, multihost–multiparasite communities are the norm [3,10,11], and both the emergence and disappearance of disease epidemics will thus be shaped by how these complex communities influence disease transmission [4]. We developed a metric for quantifying overall parasite transmission potential, $\beta(\sigma/\tau)$, which we then applied to a natural multihost–multiparasite system. We found that both a bacterial and a fungal parasite can spill over from reservoir (*Daphnia*) hosts to an alternative (*Ceriodaphnia*) host. While spillover was low for both parasites, we nevertheless uncovered important differences between the bacterium and fungus that will shape disease epidemiology as well as the evolution of transmission and virulence in this community.

Care must be taken when comparing the consequences of spillover for the two parasites, as each parasite was examined in a separate experimental block. It is nevertheless clear that there are qualitative differences in the relative importance of interspecific and intraspecific host variation for transmission potential of the bacterium and the fungus. All *Daphnia* lines suffered at least one bacterial infection, but only three of eight *Ceriodaphnia* lines suffered bacterial infection, and prevalence was low in those three susceptible *Ceriodaphnia* lines (figure 1*a*). Spillover was greater (and

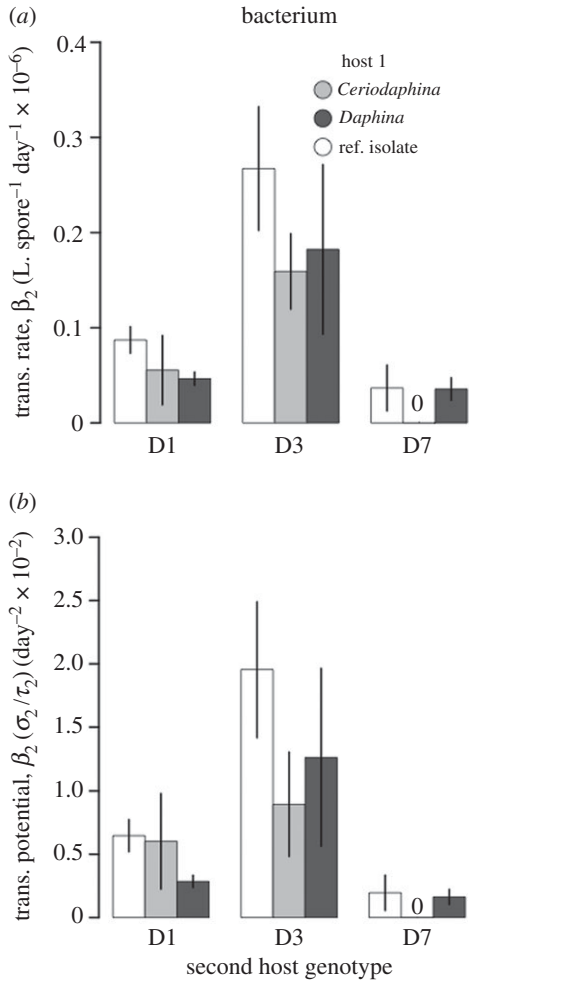

Figure 7. (*a*) Parasite transmission rate, and (*b*) overall parasite transmission potential in three *Daphnia* genotypes for bacteria (*P. ramosa*) that had passed through either the spillover host, *Ceriodaphnia*, the reservoir host, *Daphnia*, or had not passed through a host (reference isolate).

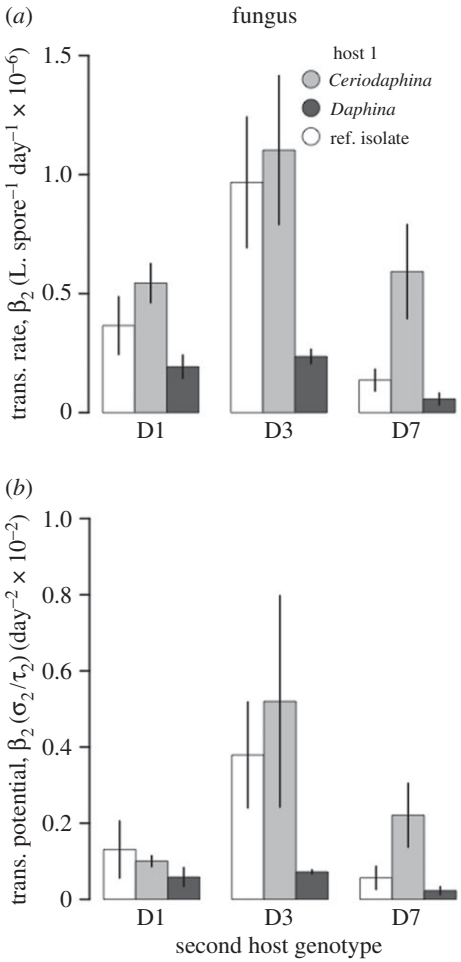

Figure 8. (*a*) Parasite transmission rate, and (*b*) overall parasite transmission potential in three *Daphnia* genotypes for fungus (*M. bicuspidata*) that had passed through either the spillover host, *Ceriodaphnia*, the reservoir host, *Daphnia*, or had not passed through a host (reference isolate).

therefore dilution was lower) for the fungus: all *Ceriodaphnia* lines were susceptible, though overall disease prevalence was lower than in *Daphnia* (consistent with an earlier study [13]; figure 2*a*). These differences in transmission patterns might be due to how the two parasites infect their hosts. The *Pasteuria* bacterium is highly specialized to small suites of host genotypes: for multiple Cladoceran host species, infection depends on the precise combination of host genotype and parasite line (that is, there is genotype specificity: [14,23–25]). In this community, it appears most *Pasteuria* genotypes collected from *Daphnia* can infect only *Daphnia*, but a small subset of strains can infect both *Ceriodaphnia* and *Daphnia* genotypes. By contrast, the fungus *Metschnikowia* is a generalist: infection depends principally on exposure to the host, which is largely governed by host feeding rate [16,26]; there is no evidence for genotypic specificity in the fungus [27,28]. Unfortunately, we did not have field-collected infected *Ceriodaphnia* to work with for this experiment. A future experiment exploring intra- and interspecific transmission of field-collected, *Pasteuria*-infected *Ceriodaphnia* would be valuable for helping to determine the roles of genotype specificity and host quality on patterns of transmission of this parasite.

The replication of parasite transmission stages within the host followed a similar pattern to parasite infectivity: for both parasites, fewer spores were produced in spillover than in reservoir hosts (figures 1*b* and 2*b*), resulting in vastly reduced overall transmission potential (figures 1*c* and 2*c*). However, there were also qualitative differences between the bacterium and the fungus for patterns of virulence (i.e. harm done to infected hosts): infection with the specialist bacterium led to reduced host survival in the spillover *Ceriodaphnia* host, but extended survival in the focal *Daphnia* host (figure 3*a*); by contrast, the fungus was equally virulent to both *Ceriodaphnia* and *Daphnia* in terms of survival (figure 5*a*). The bacterium caused similar reductions in fecundity in *Ceriodaphnia* and *Daphnia* (figure 3*b*), as did the fungus. Infection status (infected or not) explains most of the variation in host fitness for bacterium- and fungus-exposed hosts. However, in hosts where fungal infection was established, there was a positive relationship between measures of host and parasite fitness; here, host genotypes that were able to live longer when infected by the fungus were able to produce more babies and also more parasite spores.

While prevalence in the spillover host is likely to be low for both parasites, the predictability of spillover events will likely differ between the bacterium and fungus. Bacterial spillover events depend strongly on the density of a specific suite of *Ceriodaphnia* genotypes, i.e. the bacterium has a very small effective range in the spillover host [29]; this reduces the likelihood of a spillover event. By contrast, the fungus's relative generalism makes spillover more likely. The fungus may thus be a candidate for being more of a stable multihost parasite than the bacterium. The very low bacterial transmission to

Ceriodaphnia means there will have been little opportunity for adaptation, which can explain the reduced parasite growth on the spillover host. Moreover, if optimal virulence in the reservoir host differs substantially from that in the spillover host, bacterial adaptation to the more abundant reservoir host may have directly led to maladaptation to the spillover host [1,7].

There may be some benefit of high virulence in the spillover host for the bacterium, but only under very specific conditions. Previous research has demonstrated that predation of infected *Daphnia* can reduce disease when the parasite has not had sufficient time to reach maturity (and become infectious), and that predation of hosts infected with the slow-developing bacterium may explain why the rapidly developing fungus dominates in many natural systems [18]. Under high predation environments, *Pasteuria* that can infect *Ceriodaphnia* may be at an advantage as its rapid development within the spillover host means it is more likely to successfully complete its infection (life) cycle than *Pasteuria* that infects *Daphnia* only (even though total spore production is lower). However, in many cases, it seems that any bacterial fitness benefits resulting from infecting the spillover host in the presence of host predators will be negated by the fitness costs of generally low overall transmission potential.

The long-term consequences of parasite spillover in a multihost system will depend on the rate of transmission from the spillover host back to the original reservoir host. Low levels of transmission back to the reservoir host would show spillover hosts to be transmission 'dead-ends' that ultimately dilute the parasite from the reservoir host population. Conversely, high levels could fuel epidemics in the reservoir host. In experiment 2 of this study, we found evidence for transmission from the spillover host back to the original reservoir host for both bacterial and fungal parasites. Transmission of the bacterium from *Ceriodaphnia* to *Daphnia* was no different than transmission between *Daphnia* (figure 7). However, transmission of the fungus from *Ceriodaphnia* back to *Daphnia* was significantly higher than transmission rate between *Daphnia*, though overall transmission potential was not significantly different (figure 8). While the reasons for this remain to be explored, it is possible that this is due to plastic effects of host quality on *Metschnikowia* spores, as has been seen for different genotypes of *Daphnia* [28]. Thus, *Ceriodaphnia* is not a dead-end host for either parasite, and transmission from this spillover host back to the reservoir host could potentially augment epidemics in *Daphnia*, particularly for the fungus.

5. Conclusion

Truly single host–single parasite systems are rare, and so community context is key in understanding patterns of disease. However, the complexity of most natural multihost–multiparasite communities makes measuring parasite transmission enormously challenging. We quantified spillover and transmission back to the original host for two very different parasites (a specialist bacterium and a generalist fungus) in a natural host–parasite community. We argue that the relative generalism of the fungus makes it more likely to persist as a stable multihost parasite in the long-term than the specialist bacterium, which we instead expect to see in rare spillover events. Transmission back to the original host was high for both parasites, indicating that while inferior, the spillover host is not a dead-end for either parasite. Differences in parasite virulence across host and parasite combinations showed how prevalence is an incomplete metric for parasite transmission capability. Our metric for overall transmission potential, which incorporates both parasite transmission rate and parasite growth rate, allows a more useful comparison between different parasites within a community.

Data accessibility. Data and code are deposited at Dryad: doi:10.5061/dryad.3jm7h.

Author's contributions. S.K.J.R.A. and M.A.D. designed the experiment; S.K.J.R.A. and C.L.S. collected the data; S.K.J.R.A. analysed the data; S.K.J.R.A., C.L.S. and M.A.D. wrote the manuscript.

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Competing interests. We declare we have no competing interests.

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