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Inference of parasite local adaptation using two different fitness components

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local adaptation;
microsporidia;
parasite;
virulence.

Abstract

Estimating parasite fitness is central to studies aiming to understand parasite evolution. Theoretical models generally use the basic reproductive rate R_0 to express fitness, yet it is very difficult to quantify R_0 empirically and experimental studies often use fitness components such as infection intensity or infectivity as substitutes. These surrogate measures may be biased in several ways. We assessed local adaptation of the microsporidium *Ordospora colligata* to its host, the crustacean *Daphnia magna* using two different parasite fitness components: infection persistence over several host generations in experimental populations and infection intensity in individual hosts. We argue that infection persistence is a close estimator of R_0, whereas infection intensity measures only a component of it. Both measures show a pattern that is consistent with parasite local adaptation and they correlate positively. However, several inconsistencies between them suggest that infection intensity may at times provide an inadequate estimate of parasite fitness.

Introduction

Host–parasite coevolution in a spatially structured environment can result in local adaptation of the parasite to its host or vice versa. Cross-infection experiments that compare sympatric and allopatric host–parasite combinations will reveal which antagonist is ahead in the arms race and thus locally adapted to its opponent (Gandon & van Zandt, 1998; Kawecki & Ebert, 2004). Studies have found evidence for local adaptation of the parasite, the host and neither of the two (Ebert, 1994; Imhoof & Schmid-Hempel, 1998; Kaltz *et al.*, 1999; Lively & Dybdahl, 2000; Mutikainen *et al.*, 2000) and two hypotheses have been proposed that can explain these results. Firstly, the speed of adaptation is likely to be limited by the available genetic variation, and therefore it is the relative evolutionary potential of the two antagonists, i.e. how they compare in mutation and migration rates, population sizes and generation times, which determines which will be locally adapted (Gandon *et al.*, 1996; Gandon & Michalakis, 2002; Morgan *et al.*, 2005). Secondly, the coevolutionary process between the two antagonists occurs with a time lag, and even antagonists which are highly adapted will at times appear non- or even maladapted (Morand *et al.*, 1996; Kaltz & Shykoff, 1998).

Above all, it is crucial to obtain an unbiased estimate of parasite fitness if parasite local adaptation is to be studied. A parasite that is adapted to its host is assumed to have evolved an exploitation strategy that optimizes its fitness, which is frequently given as its lifetime reproductive success, i.e. the basic reproductive rate R_0. R_0 equals the number of secondary infections caused by an infected host introduced into a population of susceptible hosts. Only parasites with an $R_0 > 1$ can invade a host population and maintain a persistent infection (Anderson & May, 1981).

Empirically, it is very difficult to obtain an unbiased measure of parasite fitness and fitness components are therefore used as an approximation (Ebert, 1994; Ebert *et al.*, 1998; Imhoof & Schmid-Hempel, 1998; Kaltz *et al.*, 1999; Lively & Dybdahl, 2000; Mutikainen *et al.*, 2000; Thrall *et al.*, 2002; Cory & Myers, 2004). Intensity of infection, the number of parasites that a host carries, has

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often been used as a surrogate of parasite fitness, as it is easy to estimate and its relation to transmission seems plausible (but hardly ever tested, but see Keroack & Fields, 1986; Ebert, 1994, 1998; Hochberg, 1998; Mackinnon & Read, 1999).

Using surrogates of fitness bears the danger of providing spurious support for hypotheses about parasite evolution, e.g. for a positive correlation between parasite fitness and virulence (Hochberg, 1998; Dybdahl & Storfer, 2003). Results may be misleading for various reasons: firstly, different fitness components may trade off against each other and selection on both traits may be balancing, rather than directional. For example, virulence and transmission stage production, both components of R_0, have been suggested to be closely linked to each other (Levin & Pimentel, 1981; Anderson & May, 1982; Ebert & Herre, 1996). The parasite genotype with the lowest virulence or the highest transmission rate will not necessarily be the fittest (Jensen *et al.*, 2006). Another trade-off can arise between quality and quantity of transmission stages, and at times the production of few but qualitatively superior propagules may be a better strategy (De Paepe & Taddei, 2006). Secondly, a host is likely to offer a repertoire of specific and nonspecific defense components (Schmid-Hempel & Ebert, 2003; Mucklow *et al.*, 2004) and if the measure of parasite performance in question omits a specific component, important differences between parasite genotypes may be missed. Thirdly, if different selective forces act during the dissemination of the infection within a host, the fitness component that is measured may have no bearing on parasite fitness on the level of the host population (Levin & Bull, 1994).

Because the current theoretical framework that explains parasite and host local adaptation can accommodate for any result, it is particularly important to verify the suitability of fitness surrogates in these studies. Here, we have examined local adaptation of the microsporidian parasite *Ordospora colligata* (Larsson *et al.*, 1997) to its host, the freshwater crustacean *Daphnia magna* Straus, and aimed at verifying the usefulness of infection intensity by correlating it with a less biased fitness measure of the parasite, infection persistence.

We chose infection intensity [sometimes called parasitemia (Mackinnon & Read, 1999), parasite load (Pulkkinen & Ebert, 2004; Ganz & Washburn, 2006) or parasite burden (Koskela *et al.*, 2001)] because it has been frequently used as a measure of parasite performance in relation to fitness, including *Daphnia* and different microparasites. We chose infection persistence, the ability of the parasite to maintain a permanent infection in an experimental host population, because we think that persistence is a less biased estimator of parasite fitness than individual fitness components such as spore production and virulence. Host evolution was excluded by the use of genetically uniform populations of an asexually reproducing host. Therefore, our measure of persistence is linked to the parasite's reproductive rate in a specific host genotype because only parasites with $R_0 > 1$ can persist (Anderson & May, 1981). R_0 measures parasite fitness at the level of the host population and encompasses a broad range of biological aspects, including within and between host processes that are thought to play key roles in host–parasite interactions. Thus, estimates of parasite persistence circumvent some of the pitfalls of individual fitness components. We show that both fitness components, infection persistence and infection intensity, indicate parasite local adaptation, yet several inconsistencies between the two measures caution that infection intensity at times may yield inadequate estimates of parasite fitness.

Material and methods

The host–parasite system

Daphnia magna Straus is a small (< 5 mm) freshwater crustacean of eutrophic ponds and lakes. It reproduces by cyclical parthenogenesis and can be raised clonally in the laboratory. At 20 °C and good feeding conditions, maturity is reached after approximately 10 days and clutches of clonal offspring are released every 3–4 days thereafter. We kept animals in artificial culture medium (Klüttgen *et al.*, 1994, medium modified as explained in http://evolution.unibas.ch/ebert/lab/adam.htm) and fed the unicellular green algae *Scenedesmus* sp. With constant food supply, *Daphnia* populations reach a more or less stable equilibrium population size and can be kept in this way for many generations.

We used 12 *D. magna* genotypes, three from each of four populations: Cumnor (near Oxford, UK; genotypes C1-3), Heverlee (near Leuven, Belgium; H1-3), Kniphagen (near Plön, Germany; K1-3) and Ladykirk (80 km southeast of Edinburgh, UK; L1-3).

Ordospora colligata is a microsporidian parasite of *D. magna* (Larsson *et al.*, 1997). It infects the gut epithelium of its host where it multiplies and eventually undergoes sporogony. Development from infection to the release of new infectious spores takes about 3 days. Spores are released into the environment via the faeces and transmit horizontally. At the same time, they disseminate in the gut epithelium via autoinfection, strongly proliferate within their host and lead to a chronic infection (Mangin *et al.*, 1995, where this parasite has been named *Pleistophora intestinalis*). The parasite reduces fecundity and increases mortality of its host by about 5–20% (Ebert *et al.*, 2000), it has been shown to drive evolution in populations of *D. magna* (Capaul & Ebert, 2003; Haag & Ebert, 2004; Ebert, 2005), and it can reach high prevalences in natural populations (Decaestecker *et al.*, 2005).

We used seven isolates of *O. colligata*. Isolates C, H, K and L originated from the corresponding *Daphnia* populations mentioned above (the original hosts are

genotypes C1, H1, K1 and L1 respectively). Isolate Ka comes from Kaimes (UK), isolates T1 and T2 from the Tvärminne archipelago in southern Finland. The parasite isolates cannot be cultured outside their *Daphnia* host. Therefore, isolates were kept in monoclonal populations of their original host genotype. These stocks were used to obtain spores or animals serving as spore donors to carry out infections.

Experiments were carried out under constant temperature (20 ± 1 °C) and a 16 : 8 hours light: dark cycle. All hosts were cured prior to the experiments by isolation of embryos from the brood pouch of their mother, which prevents horizontal infection [see Ebert (2005) for details]. Absence of the parasite was verified by phase contrast microscopy at 400× magnification and PCR [using the protocol of Refardt & Ebert (2006)].

Experiment 1: infection persistence in monoclonal host populations

We established monoclonal populations from each of the 12 *Daphnia* genotypes by placing five adult hosts in a 100-mL jar filled with 80mL medium. These populations were then exposed to spores of parasite isolates (C, H, K, L and T1) or were left unexposed. Every host genotype–treatment combination was replicated eight times yielding a total of 12 host genotypes × 6 treatments (=5 parasite isolates plus controls) × 8 replicates = 576 experimental populations. Populations were exposed to the parasite on day 3 (1000 spores per population), days 5 and 6 (1500 spores each) and day 24 (6000 spores). Populations were fed three times a week with 1.5×10^7 algae cells and the water was changed during days 64–67 of the experiment.

Populations that went extinct during the course of the experiment were recolonized by adding four uninfected animals of the same host genotype from a stock culture. Because parasite spores can survive the extinction of their hosts for some time, we expected that newly added hosts would become infected again. By doing so, we allowed parasites to persist even if they caused host population extinction. We chose this approach because *O. colligata* has a comparatively low virulence and is unlikely to cause host population extinction in larger experimental populations (Ebert *et al.*, 2000). Taking extinctions into account for the measurement of persistence would not be meaningful here.

The experiment was terminated during days 115–124 when the biggest animal from every experimental population was dissected and examined for signs of infection (phase contrast microscope, 400× magnification). Sampling order was random. Inferring parasite presence in a population by checking a single adult animal is reliable for this parasite because infection intensity increases with age and the oldest and biggest animals are the main carriers of parasites in a population (D. Refardt, pers. obs.; Stirnadel & Ebert, 1997).

Experiment 2: infection intensity in individual hosts

We placed newborn animals of all 12 host genotypes singly in 1.5mL medium and exposed them to a suspension of spores (5000 spores in 100μL water) of a parasite isolate (C, H, L, Ka, T1 and T2). Controls were left unexposed. Broods were split across treatments. Every host genotype–treatment combination was replicated eight times yielding a total of 12 host genotypes × 7 treatments (=6 parasite isolates plus controls) × 8 replicates = 672 animals. Animals were fed daily with 1.5×10^6 algae cells until day 4 and 2×10^6 algae cells thereafter. Newborns were removed daily and animals were placed into new medium on day 8 of the experiment. On day 17, the experiment was terminated. All animals were placed singly in 1.5-mL tubes and stored at −80 °C.

To measure infection intensity, total genomic DNA was extracted from all animals and parasite DNA was quantified by qPCR as described in Refardt & Ebert (2006). Quantification of parasite DNA was done relative to a standard with a known concentration (target sequence cloned into a plasmid, 2×10^4 and 2×10^5 copies) and hence is given as copy number of the target sequence.

Statistical analyses

Infection persistence was analysed with ordinal logistic regression (dependent variable: proportion of replicates per host genotype–parasite isolate combination in which the parasite persisted). Infection intensity was $\log_{10}(x + 1)$ transformed and analysed with analysis of variance (ANOVA). Host genotypes were nested in host population and all factors were treated as random. Relationships between different response variables obtained in the experiments were explored with non-parametric tests. All statistical analyses were done with JMP v. 5.0.1a (SAS Institute Inc., Cary, NC, USA).

Significant parasite isolate × host population and parasite isolate × host genotype interactions may be due to local adaptation. To investigate this further, we carried out least square means contrasts on the infection intensity data. Contrasts were specified to compare either parasite isolates within the same host population (local vs. foreign criterion of local adaptation), or to compare the same parasite isolate in different host populations (home vs. away criterion of local adaptation; Kawecki & Ebert, 2004).

Results

Experiment 1: infection persistence in monoclonal host populations

All parasite isolates persisted well in populations of the host genotype they were originally isolated from, which indicates that experimental conditions allowed persistent

Table 1 Number of replicate populations (out of eight) in which parasite infections persisted throughout 3 months (about 6–8 host generations) in Experiment 1. Sympatric host–parasite combinations are set in bold font with the host genotype underlined in which the parasite isolate was found originally.

	Host population and host genotype											
	Cumnor			Heverlee			Kniphagen			Ladykirk		
Ordospora colligata isolate	C1	C2	C3	H1	H2	H3	K1	K2	K3	L1	L2	L3
Cumnor	**6**	**4**	**3**	0	0	0	0	0	0*	0	0	0
Heverlee	4	4	8	**7**	**0**	**0**	0	0	0	0	0	0
Kniphagen	5	4	8	1	0	0	**7**	**8**	**8**	1	0	0
Ladykirk	6	7	8	0	0	0	0	0	0	**6**	**2**	**8**
Tvärminne 1	0	0	3	0	0	0	0	0	0	0	0	0

*Only seven replicates.

Table 2 Ordinal logistic regression of infection persistence data of *Ordospora colligata* isolates (Experiment 1). Replicated host populations were screened for the presence of the parasite 3 months after exposure and the proportion of replicates of every host genotype–parasite isolate combination in which the infection persisted was used as a measure of persistence.

Source	d.f.	L-R χ^2	$P > \chi^2$
Parasite isolate	4	21.78	< 0.001
Host population	3	32.65	< 0.001
Parasite isolate × host population	12	36.41	< 0.001

infections of natural host–parasite combinations (Table 1). One replicate of genotype K3 infected with isolate C was excluded from the analysis of infection persistence because its infection status could not be determined unambiguously. The results for infection persistence were determined by both strong main effects and strong parasite isolate × host population interactions (Tables 1 and 2). The latter were mainly caused by the ability of parasites to persist in their sympatric hosts. In hosts from Heverlee, Kniphagen and Ladykirk, sympatric parasite isolates were able to establish persistent infections almost exclusively (Table 1). Exceptions from this pattern were the three host clones from Cumnor. These clones allowed (with some variation) persistent infections of all isolates (Table 1).

Twenty-one of the 575 experimental populations (3.7%) went extinct during the 115 days of the experiment. None of the 96 control populations went extinct, which suggests exposure to parasites as cause of extinction (Fisher's exact test for proportions of extinctions in control and treated populations, $P_{\text{two tailed}} = 0.036$). Infection persistence and extinctions correlated positively across all host genotype–parasite isolate combinations (Fig. 1, $n = 60$, Spearman's $\rho = 0.32$, $P = 0.012$), suggesting that good persistence was correlated with high parasite virulence. We recolonized extinct populations with uninfected individuals which were subsequently infected by parasite spores that were still present. If the parasite persisted, we scored these replicates as persistent infections. None of the results was changed when these replicates were scored as nonpersistent.

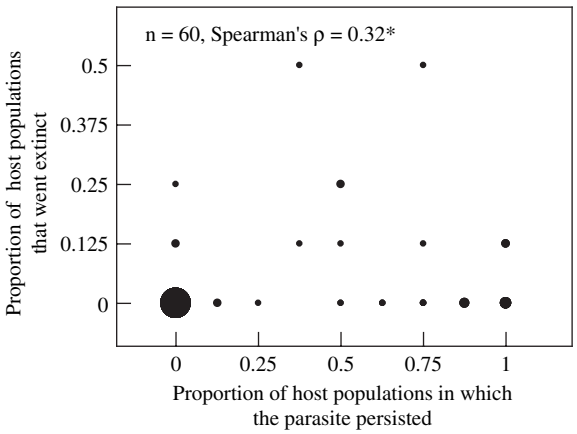

Fig. 1 Proportion of replicate host populations that went extinct during the course of Experiment 1 plotted against the proportion in which the parasite maintained a persistent infection. Extinct populations were recolonized, allowing the parasite to persist despite host population extinction. Data that overlay on the plot are visualized by a proportional increase of the area of the respective marker.

Experiment 2: infection intensity in individual hosts

Twenty-two of 672 (3.3%) animals died during the course of the experiment and two were lost because of handling error. Controls (where two deaths occurred) are not considered in the analysis of infection intensity, leaving 554 animals. Deaths occurred almost exclusively in host genotypes C1, C2 and C3 from Cumnor where 6, 2 and 11 animals died respectively.

Average infection per host genotype–parasite isolate combination was calculated including replicates in which no parasite DNA was detected by qPCR (zero readings). This was done for the following reason: zero readings occurred more often in replicates of combinations with

otherwise low infection intensities ($n = 72$, Spearman's $\rho = 0.77$, $P < 0.001$). We have shown previously that when only 1% of the DNA extract is subjected to qPCR, false negatives can occur because of sampling error (Refardt & Ebert, 2006). This was taken into account by calculation of the average including zero readings. For this same reason, we will not report infectivity data (proportion of infected replicates). However, all analyses were also performed with infection intensity calculated as the average of only those replicates in which parasite DNA was detected, and yielded the same results qualitatively.

We found considerable variation in infection intensity between different host genotype–parasite isolate combinations (Fig. 2). Nearly 60% of the total variance in infection intensity of parasite isolates was attributable to host population (Table 3). Parasite isolates did not differ in their overall infection intensity, yet significant interactions indicated that genetic variation was present in both host genotypes and parasite isolates (Table 3). The interactions were further analysed for patterns of local adaptation using contrasts. They were either carried out between parasite isolates for separate hosts or between hosts for separate parasite isolates [according to the 'local vs. foreign' and 'home vs. away' criteria of Kawecki & Ebert (2004)].

Local vs. foreign

In hosts from Cumnor and Heverlee, sympatric parasite isolates established significantly stronger infections than allopatric isolates (Fig. 3; Cumnor: $F_{[1,40.2]} = 15.21$, $P < 0.001$; Heverlee: $F_{[1,40.2]} = 24.46$, $P < 0.001$), but this pattern was not found for hosts from Ladykirk ($F_{[1,40.2]} = 0.26$, $P = 0.61$). The results were confirmed when isolates were compared in separate host genotypes from the same population: the Cumnor isolate had the strongest infection intensity of all isolates in all three host genotypes from Cumnor (C1: $F_{[1,482]} = 11.55$, $P < 0.001$; C2: $F_{[1,482]} = 11.75$, $P < 0.001$; C3: $F_{[1,482]} = 8.57$, $P = 0.004$) and so did the Heverlee isolate in two host genotypes from Heverlee (H1: $F_{[1,482]} = 25.83$, $P < 0.001$; H2: $F_{[1,482]} = 33.93$, $P < 0.001$; H3: $F_{[1,482]} = 2.02$, $P = 0.16$). The Ladykirk isolate did not perform better than allopatric isolates in any host genotype from Ladykirk (L1: $F_{[1,482]} = 0.63$, $P = 0.43$; L2: $F_{[1,482]} = 0.09$, $P = 0.77$; L3: $F_{[1,482]} = 0.03$, $P = 0.86$).

Home vs. away

A superior performance of all parasite isolates in host genotypes from Cumnor caused a strong host population main effect (Fig. 2). Apparently, host genotypes from Cumnor are generally more susceptible. This main effect

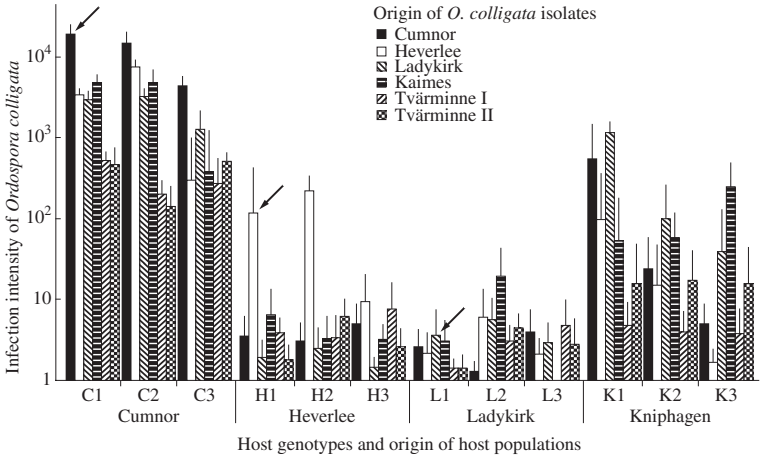

Fig. 2 Infection intensity of six isolates of *Ordospora colligata* in each of 12 *Daphnia magna* host genotypes (Experiment 2). *Ordospora colligata* isolates H, C and L encountered their sympatric host genotypes, with the arrow indicating the host genotype from which the parasite isolate was obtained originally. Error bars are 1 SEM.

Table 3 Random effects ANOVA table for infection intensity of *Ordospora colligata* isolates in different genotypes of *Daphnia magna* (Experiment 2).

Source	d.f. num	MS num	d.f. den	MS den	*F*-ratio	$P > F$	% σ^2 expl.
Parasite isolate	5	7.10	15.01	5.21	1.36	0.292	0.9
Host population	3	188.14	16.09	7.17	26.22	< 0.001	57.9
Host genotype (host population)	8	3.29	40.18	1.32	2.49	0.027	1.9
Parasite isolate × host population	15	5.22	40.23	1.32	3.95	< 0.001	7.5
Parasite isolate × host genotype (host population)	40	1.32	482	0.64	2.08	< 0.001	3.9
Error	482	0.64					

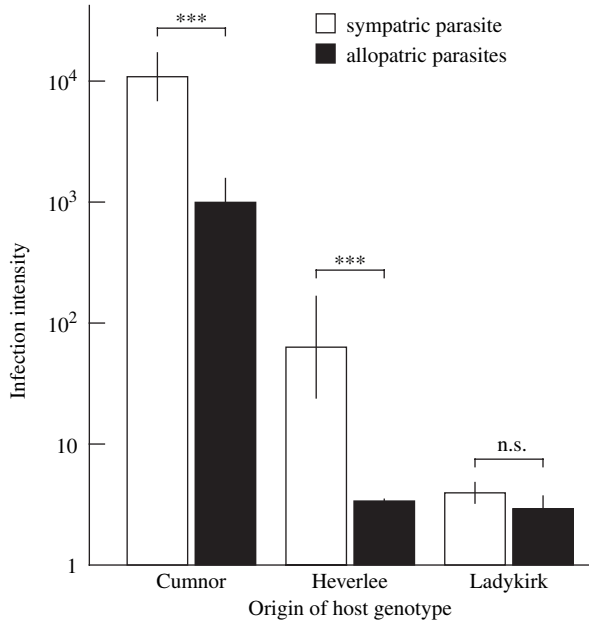

Fig. 3 Average infection intensities of *Ordospora colligata* isolates in sympatric and allopatric host–parasite combinations (Experiment 2). The comparison of parasite isolates within host populations follows the 'local vs. foreign' criterion. Infection intensities were compared with least square means contrasts. Error bars are 1 SEM.

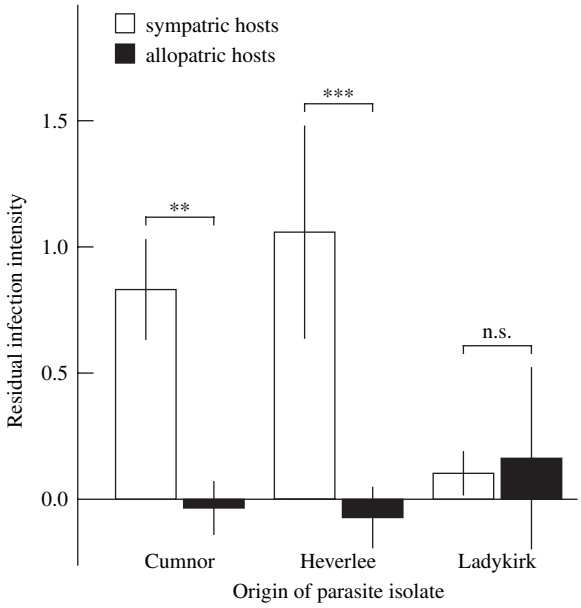

Fig. 4 Residual infection intensities (host population main effect has been removed) of *Ordospora colligata* isolates in sympatric and allopatric host–parasite combinations (Experiment 2). The comparison of infection intensities of a parasite isolate between host populations follows the 'home vs. away' criterion. Infection intensities were compared with least square means contrasts. Error bars are 1 SEM.

obscured the pattern for local adaptation. Therefore, an ANOVA was fitted to residual infection intensity after statistical removal of the host population main effect. Residual infection intensity of parasite isolates was significantly higher in sympatric hosts for isolates from Cumnor and Heverlee (Fig. 4; Cumnor: $F_{[1,40.2]} = 9.82$, $P = 0.003$; Heverlee: $F_{[1,40.2]} = 17.21$, $P < 0.001$) whereas they did not differ for the Ladykirk isolate ($F_{[1,40.2]} = 0.05$, $P = 0.83$). Thus, both criteria for local adaptation gave consistent results.

Additionally, we compared infection intensity of parasite isolates on the host genotype they were originally isolated from against the two other host genotypes from the same population. We found no evidence for a difference in infection intensity (C: $F_{[1,482]} = 1.17$, $P = 0.28$; H: $F_{[1,482]} = 1.41$, $P = 0.24$; L: $F_{[1,482]} = 0.03$, $P = 0.87$).

Comparing infection persistence and infection intensity

We correlated infection persistence from Experiment 1 with infection intensity from Experiment 2 and found a significant positive correlation (Fig. 5; $n = 48$, Spearman's $\rho = 0.53$, $P < 0.001$). When correlations were done per parasite isolate, they remained significantly positive for isolates C and H (C: $n = 12$, $\rho = 0.76$, $P = 0.004$; H: $n = 12$, $\rho = 0.72$, $P = 0.009$) and positive (yet not significantly) for isolates L and T1 (L: $n = 12$, $\rho = 0.43$, $P = 0.17$; T1: $n = 12$, $\rho = 0.39$, $P = 0.21$). Thus, on average, parasites, which established higher infection intensities, persisted longer in the small experimental host populations. However, some combinations deviated strongly from this, showing good persistence, despite of reaching only very low intensities or did not persist despite strong intensities.

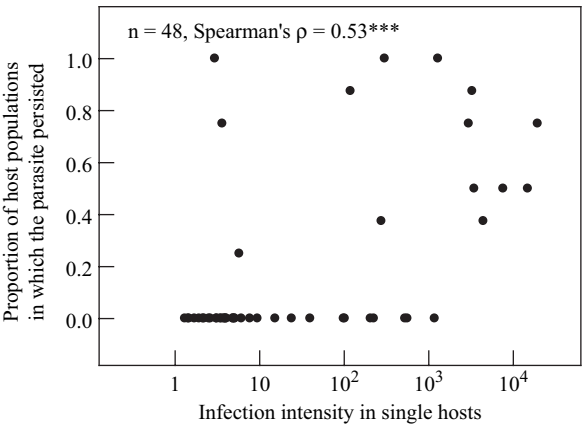

Fig. 5 Proportion of replicate host populations in which the parasite maintained a persistent infection (Experiment 1) plotted against average infection intensity in single hosts (Experiment 2). Forty-eight host genotype–parasite isolate combinations are shown.

Discussion

We quantified two different measures of parasite fitness in sympatric and allopatric combinations of host genotypes and parasite isolates sampled from different populations. We applied two criteria to infer local adaptation (Gandon & van Zandt, 1998): different parasites can be compared within the same host (local vs. foreign parasites, LvF) or a parasite can be compared between different hosts (parasite at home vs. away, HvA). It has been proposed that the LvF criterion is sufficient to infer local adaptation (Kawecki & Ebert, 2004).

Generally, our data on infection persistence give a very clear picture of local adaptation and in three of four populations both criteria yield the same result (Table 1, Heverlee, Kniphagen, Ladykirk). However, according to the LvF criterion, the parasite isolate from Cumnor is not locally adapted whereas it is according to the HvA criterion. We propose that the latter inference is not justified. The resistance of allopatric hosts to the Cumnor parasite isolate does not allow the conclusion that this isolate has evolved a specific adaptation to its sympatric host.

Analysing the data of infection intensity, two of three parasite isolates (Cumnor and Heverlee) were found to be locally adapted according to the LvF criterion. A strong host population main effect did not allow the application of the HvA criterion on the raw data. After the statistical removal of this effect, both criteria were consistent. We take this as evidence that the microsporidian parasite *O. colligata* is locally adapted to its host *D. magna*.

To our knowledge, this is the first study that uses infection persistence to investigate parasite performance. Although we think that persistence is a better fitness measure than some of the traits we used in earlier experiments with *Daphnia* parasites, it still has some drawbacks. Firstly, persistence measures parasite fitness as a binary trait and does only indicate whether a parasites' R_0 is above or below unity. The quantitative aspect seen in our experimental data is likely to be caused by stochastic effects, in particular for those combinations in which R_0 is around 1. Secondly, persistence is likely to be dependent on the environmental conditions, such as population size and density and container size. Different experimental conditions could lead to much higher or lower proportions of persisting infections, up to a stage where there is not enough variance in the data for a meaningful interpretation. Thirdly, persistence is a crucial aspect of parasite fitness in isolated populations. However, in a metapopulation, where migration between subpopulation occurs, selection may favour parasites that will not persist locally (Kerr *et al.*, 2006). This is even more so in cases where the parasite can form resting spores which allow surviving a temporary absence of the host. Of our parasite isolates, only the Finnish samples (Tvärminne) come from a metapopulation, the other are from permanent ponds. Finally, persistence as measured here estimates R_0 for specific host genotype–parasite isolate combinations. We do not suggest that our measure is comparable with parasite persistence in a natural, genetically variable host population.

In the second experiment, we measured infection intensity, which is a composite measure of within-host growth rate and infectivity. Disentangling the relative contributions of these two components is difficult (see Hochberg (1998) and Ebert (1998) for a discussion): from infection by a single spore of *O. colligata* to the release of spores takes approximately 3 days and a typical gut infection is the result of repeated autoinfections (the parasite spreads from cell to cell within the gut or an animal ingests spores it has shed itself). Thus, both infectivity of single spores as well as the length of one infectious cycle from infection to release of spores determine how quickly an infection builds up in the gut of a *Daphnia*.

Infection intensity is a measure of within-host performance of the parasite that is often used to approximate parasite fitness. It is generally easier to obtain than infection persistence (e.g. because host generations are too long). In many cases, it is reasonable to expect that infection intensity (or any other measure of within-host performance) is positively correlated with parasite fitness because successful growth in the host leads to the production of numerous propagules which are likely to enhance parasite transmission. The results of our study confirm this. We find that in this host–parasite system, persistence and intensity are correlated, which suggests that the spread of *O. colligata* is enhanced by the production of numerous propagules.

Upon closer inspection, the correlation between infection intensity and infection persistence is not tight and in several cases intensity is a very poor predictor of persistence. For example, isolate H in host H2 or isolate L in host K1 built up a considerable parasite load yet did not establish persistent infections. Likewise, isolate L in hosts from Ladykirk persisted well despite having rather low infection intensities. These discrepancies suggest that either one or both of our fitness estimates provide at times an inadequate picture of parasite fitness.

Several reasons may be put forward to explain why high infection intensity may not lead to a persistent infection. Firstly, hosts may have been exposed to an unnaturally high spore dose which allowed the parasite to infect otherwise resistant hosts (Dematteis *et al.*, 2003), yet spores shed by this host were too few to cause secondary infections. Secondly, resistance in the host population can increase after initial exposure to the parasite. It has been shown that *Daphnia* that have been exposed to the bacterial pathogen *Pasteuria ramosa* equip their offspring with specific immunity towards this bacterium (Little *et al.*, 2003). Thirdly, crowding can trigger increased investment into immune functions (Wilson & Reeson, 1998) and experimental populations, which were kept at a high density, may therefore have a higher resistance than single animals. Thus, the fitness

components measured in this study may overestimate (persistence) and/or underestimate (intensity) parasite resistance. Fourthly, it may be possible that more than one solution exists to reach a high persistence. If different fitness components combine in nonlinear ways, there may be alternative ways to reach high persistence.

Persistent infections despite low infection intensity are somewhat surprising and more difficult to explain. We propose the following: upon first exposure and successful infection, parasite load in *O. colligata* increases exponentially in the host and most likely reaches a plateau at a later stage (Mangin *et al.*, 1995). By sampling infected animals at day 17 after exposure to the parasite, we measured infection intensity while it was still increasing. Therefore, low infection intensities in our assay may be caused by parasites that grow slowly but eventually establish a high parasite load and persist in the host population.

It has been suggested that parasite fitness cannot be increased infinitely via increasing infection intensity because vigorous within-host growth causes more damage and shortens host longevity, creating a trade-off between transmission rate and the duration of an infection (Anderson & May, 1982). We found that in host–parasite combinations with high infection persistence, the host population was also more likely to go extinct which suggests a cost of virulence for the parasite. In earlier studies, *O. colligata* was found to reduce host fecundity by about 20%, but no effect on host population density was found (Ebert *et al.*, 2000). This depends on the host genotype used. In our sample, the host genotypes from Cumnor were particularly sensitive. Yet, we note that *O. colligata* has a comparatively low virulence and the observed effect on host population extinction is likely to be enhanced by the small population size that was used in the experiment.

To conclude, using infection persistence and intensity as estimates of parasite fitness, we find that the microsporidium *O. colligata* is locally adapted to its host *D. magna*. The two fitness measures are correlated positively, yet high levels of infection intensity do not always predict persistence of the parasite. We propose that infection persistence provides a more robust measure of parasite fitness.

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