QTL mapping of a natural genetic polymorphism for long-term parasite persistence in *Daphnia* populations

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SUMMARY

Knowing the determinants of the geographic ranges of parasites is important for understanding their evolutionary ecology, epidemiology and their potential to expand their range. Here we explore the determinants of geographic range in the peculiar case of a parasite species – the microsporidian *Hamiltosporidium tvaerminnensis* – that has a limited geographic distribution in a wide-spread host – *Daphnia magna*. We conducted a quantitative trait loci (QTLs) analysis with monoclonal F_2 *D. magna* populations originating from a cross between a susceptible northern European genotype and a resistant central European genotype. Contrary to our expectations, long-term persistence turned out to be a quantitative trait across the F_2 offspring. Evidence for two QTLs, one epistatic interaction and for further minor QTL was found. This finding contrasts markedly with the previously described bimodal pattern for long-term parasite persistence in natural host genotypes across Europe and leaves open the question of how a quantitative genetic trait could determine the disjunct geographic distribution of the parasite across Europe.

Key words: resistance, QTL analysis, microsporidia, *Daphnia*, plankton, crustacea, parasite persistence.

INTRODUCTION

A hallmark of parasitism is a high degree of host specificity (Antonovics *et al*. 2013). While some parasites are found in many different hosts, most parasites are specific and only infect one or a few host species (Combes, 2001; Poulin, 2007; Poulin and Keeney, 2008). However, hosts are not monolithic entities that can be classified as either susceptible or resistant to a given parasite. Rather, variation within host species is common, often visible in differences across geographic regions. Such variation is important to consider when assessing parasite specificity and may help explain the evolution of host–parasite interactions. For example, the malaria agent *Plasmodium vivax*, which has a broad geographical distribution, is almost absent in West and Central Africa (Guerra *et al*. 2010), even though other malaria agents are abundant in these regions. Similarly, the yellow fever virus affects humans in South America and Africa but is absent in Asia, even though at least one of its vectors, *Aedes aegypti*, is found there (Rogers *et al*. 2006; Agampodi and Wickramage, 2013). These examples highlight the geographic dynamics of parasites spreading, evolving and coevolving in interaction with their hosts and the local environment. Understanding the geographic distributions of parasites within host species can provide important clues for their evolutionary ecology and epidemiology, as well as for global health issues.

Ecological and genetic factors may influence whether or not local host populations allow parasites to spread. For example, local temperature and host density are both considered important ecological factors in determining disease persistence (Dobson *et al*. 2015; Gagne *et al*. 2015; Ryan *et al*. 2015). On the other hand, in the above-mentioned malaria agent *P. vivax*, it is genetic factors that limit the parasite's spread. Genetic research suggests that resistance to *P. vivax* is linked to the absence of the Duffy antigen in most local human populations in West Africa (Culleton *et al*. 2008). For most parasites, little is known about the host genetic factors that vary across geographic space and that may influence the spread of parasites. Understanding how these genetic factors affect parasite distribution would facilitate our ability to predict the potential spread of parasites into new areas after potential ecological limits are removed, e.g. after human-induced changes, such as climate change (Huyse *et al*. 2005).

Exposure trials of individual hosts are often a starting point for experimentally identifying the host genetic factors that determine parasite geographic ranges. Common garden experiments allow us to separate out ecological factors from host genetic effects. Such experiments are particularly useful for identifying quantitative genetic traits, which are sensitive to environmental variation (Falconer and MacKay, 1996). However, while such experiments can establish whether the parasite is or is not able to infect the host, they do not answer

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the question of whether the parasite is able to persist in the host population. Successful infections do not always lead to sufficient parasite replication or allow for transmission. Only infections that can transmit to other hosts and whose basic reproductive number exceeds one ($R_0 > 1$) are predicted to persist in a host population (Wolfe *et al*. 2004; Woolhouse and Antia, 2008). However, to test whether R_0 exceeds one requires population-level experiments, which are, unfortunately, often not possible to set up [but see Refardt and Ebert (2007)]. Thus, our ability to determine parasite persistence in a host population is limited.

Using population-level common garden experiments, Lange *et al*. (2015) tested for genetic effects behind the strongly disjunct distribution of the microsporidian parasite, *Hamiltosporidium tvaerminnensis*, in European populations of the planktonic crustacean *Daphnia magna*. While the host species occurs all across Eurasia, North Africa and North America, the parasite has only been recorded in Fennoscandian coastal rock pools and in populations close to the Mediterranean Sea. Consistent with the geographic distribution of the parasite, the experiment revealed that host populations from geographic areas without the parasite do not allow parasite persistence, while those from within the parasite distribution were highly permissible (Lange *et al*. 2015). Parasite persistence showed a clear bimodal pattern, with hosts being either susceptible or not. These findings strongly suggested that host genetics were responsible for determining the parasite's geographic distribution. The current study was designed to further scrutinize this hypothesis. It uses quantitative trait loci (QTLs) F_2-panel to find regions in the host genome (the loci) associated with variation in parasite persistence (the quantitative trait) and to explore the genomic architecture underlying this natural polymorphism. QTL studies have been shown to be powerful tools for uncovering the genetic architecture of host resistance variation in plants and animals (Huang *et al*. 2014; Atlija *et al*. 2015; Bento *et al*. 2017).

The study system

The microsporidium *H. tvaerminnensis* (formerly misclassified as *Octosporea bayeri*, Corradi *et al*. 2009; Haag *et al*. 2011) infects the fat body and ovaries of the cladoceran *D. magna*, thereby reducing its competitive ability, fecundity and lifespan (Ben-Ami *et al*. 2008; Bieger and Ebert, 2009). In natural populations, the parasite has never been found in any host species other than *D. magna*. *Hamiltosporidium tvaerminnensis* has a mixed mode of transmission – a combination of vertical transmission (mother to offspring) and horizontal transmission [through water-borne environmental spores released from dead hosts (Vizoso and Ebert, 2005; Ebert, 2013)]. As all asexual offspring of infected mothers become infected, prevalence can quickly reach 100% in experimental and natural populations (Lass and Ebert, 2006; Lass *et al*. 2011).

Although *D. magna* is found in standing fresh and brackish water across Holarctic and North Africa, the distribution of *H. tvaerminnensis* is disjunct, with a Northern European range in rock pool populations along the Swedish and Finnish coast and in populations around the Mediterranean Sea (Goren and Ben-Ami, 2013; Haag *et al*. 2013*a*, *b*). Recently it was also found in Siberian *D. magna* populations (D. Ebert, unpublished observations). Important for the current study, *H. tvaerminnensis* has never been found in the central European populations of *D. magna*, the most-intensively studied geographic region of the host species' distribution. It seems unlikely that the disjunct range of *H. tvaerminnensis* is a consequence of limited migration, as population genetic data for the host suggest high migration rates (De Gelas and De Meester, 2005; Fields *et al*. 2015), and *H. tvaerminnensis* can survive and co-migrate in the resting stage of its host (Vizoso *et al*. 2005).

Several studies have sought to explain the disjunct geographic range of *H. tvaerminnensis*. Experimental infections of individual hosts from Finnish and German populations have revealed that spore counts are on average about two orders of magnitude higher in hosts within the native range of *H. tvaerminnensis* (Ebert, 2008), but that totally resistant host clones are rare. To investigate the genetics underlying this difference, Routtu and Ebert (2015) used a QTL approach to examine a parent clone from the Finnish home range of the parasite and one from outside this range (Germany). The authors tested clonal lines of the F_2 generation, counting the number of parasite spores after horizontal and vertical infection trials. Spore counts suggested quantitative genetic variation following both vertical and horizontal infections (Routtu and Ebert, 2015).

Since *H. tvaerminnensis* seems able to infect all *D. magna* genotypes, researchers have hypothesized that the parasite is unable to persist in *D. magna* populations where it has a poor growth rate. To test this idea, Lange *et al*. (2015) produced monoclonal host populations in a common garden experiment and exposed them to isolates of *H. tvaerminnensis* from its northern range. Parasite persistence was monitored over 25 weeks (about four to eight generations), thus allowing for several host generations and complete transmission cycles. All *D. magna* clones from the parasite's native geographic range allowed for long-term persistence, while most host clones from outside the range did not, resulting in a clear genetic bimodal infection pattern (Lange *et al*. 2015). Interestingly, the Finnish isolates of *H. tvaerminnensis* were also able to persist in *D. magna* populations from around the

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Mediterranean Sea, where the parasite is known to occur. The clear correspondence in this study between the parasite's persistence pattern and its known geographic distribution suggests that long-term persistence experiments are better than spore counts to study the genetic factors underlying the paraiste's disjunct distribution in *D. magna* populations. We therefore decided to undertake another QTL study but instead of using spore counts in individual hosts, we quantified parasite persistence in monoclonal host populations. Reinforcing the results of the spore count study (Routtu and Ebert, 2015), this study found that the host's propensity to allow long-term parasite persistence is a quantitative trait caused by few major and several minor QTLs.

MATERIAL AND METHODS

Daphnia magna is a freshwater, planktonic crustacean. It is a cyclic parthenogenetic organism that reproduces mainly asexually, although environmental conditions can trigger sexual reproduction. During sexual reproduction, females produce clonal sons and haploid resting-eggs. After fertilization, resting eggs are deposited in shells (ephippia) that can endure harsh environmental conditions, such as summer drought and winter freezing. Clonal lines can be maintained in the laboratory in artificial *Daphnia* medium (ADaM, Klüttgen *et al*. 1994) on a diet of green algae *Scenedesmus* sp.

The *D. magna* F_2 QTL panel is a standing panel of 370 F_2-lines maintained clonally in the laboratory. It has been used and described in previous publications (Routtu *et al*. 2010, 2014; Roulin *et al*. 2013). In short, two parent clones with divergent phenotypes for parasite resistance and behavioural traits were chosen and selfed. The maternal clone, Xinb3 (BB genotype) from the Tvärminne region in Finland, was selfed three times; the other parental clone, Iinb1 from Munich, Germany (AA genotype), was selfed once. Xinb3 is from the centre of the geographic range of *H. tvaerminnensis* and allows parasite persistence, whereas Iinb1 does not. The two parental clones were crossed to produce an F_1 hybrid clone, which was selfed to obtain the F_2 clones. All the F_2 clones were genotyped at 1324 SNP-markers (Routtu *et al*. 2014). The same *D. magna* QTL panel was successfully used to identify the genes responsible for resting stage induction and resistance to a bacterial pathogen (Roulin *et al*. 2016; Bento *et al*. 2017).

The current study used 200 clones from the F_2 QTL panel. Because successful vertical transmission seemed important for the parasite to persist in its host, we selected 100 F_2 clones that Routtu and Ebert (2015) had described as having recombination events close to a major QTL for spore counts after vertical transmission (±15 cM from a marker region on Linkage group 6; scaffold00606_2933). The other 100 F_2 clones were chosen randomly from among the remaining F_2 clones.

Hamiltosporidium tvaerminnensis is an obligate intracellular parasite of *D. magna* (Vizoso *et al*. 2005). The parasite isolate used here was obtained from an infected *D. magna* female collected in a rock pool on the island Ören in the Tvärminne archipelago of southwest Finland. This is the same isolate (FI-OER-3-3) whose genome was sequenced and annotated (Corradi *et al*. 2009). In the laboratory, the parasite is maintained in culture together with a clone of the founder *D. magna* female.

Infection trial

The experiment followed the protocol of Lange *et al*. (2015). Three monoclonal populations were produced for each of the 200 F_2 clones, and nine monoclonal populations were produced from the parental clones Xinb3, Iinb1 and the F_1 hybrid. These were kept in 380-mL jars. In addition to these monoclonal populations, 18 replicates were produced to serve as uninfected controls (nine for each parents). Replicates were randomly distributed and kept in an incubator set at 20 °C, 80% humidity, and a day: night cycle of 16:8 h. The positions of the jars in the incubator were changed weekly. Populations were fed 50 million cells of *Scenedesmus* sp. three times a week. All populations were reduced by one half every 5 weeks and transferred into clean jars with fresh ADaM. This reduction increased population turnover. To infect the hosts, we produced parasite spore suspensions at a concentration of 3·5 million spores mL^{-1} using *H. tvaerminnensis* FI-OER-3-3 from infected hosts homogenized in ADaM. Roughly 1 mL of this suspension was added to all jars (except controls). Control host populations were treated with a suspension made from homogenized uninfected *D. magna* clone FI-OER-3-3.

To essay if *D. magna* populations were infected, we made squash preparations of five adult *Daphnia* individuals from each replicate population. These preparations were placed on a glass slide, homogenized and stained with 14 μL of a 1:1 solution of Calcofluor White staining with 10% sodium hydroxide (NaOH). The slides were examined under UV light with a phase contrast microscope. Calcofluor White stains chitin in the spore wall of *H. tvaerminnensis*, so the spores appear light blue under UV light and are clearly visible. If at least one of the five *D. magna* from a replicate population was infected, the replicate population was counted as infected. To reduce the chance of accidentally overlooking infections, we increased the number of examined *Daphnia* to 15 for our final assessment at 30 weeks. However, this made no difference in the scoring, as replicate populations typically had either a high prevalence or were uninfected.

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QTL mapping

The 0/1 data for long-term persistence of *H. tvaerminnensis* in each replicate jar after 5 and 30 weeks were treated as proportions, i.e. infected replicates over the total number of replicates ($n = 3$) per clone. All analyses were done using the R statistical software version 3.0.2 (R Development Core Team, 2008). QTL mapping was conducted with Rqtl (version 1.34–17, Broman and Sen, 2009) as described in Routtu and Ebert (2015; see Supplementary R-script). For robustness and speed of the QTL analysis, we used Haley–Knott regression (Haley and Knott, 1992). Model assumptions were tested and confirmed. To check for epistatic interactions, a two-dimensional scan (scantwo) was run. Further, candidates were analysed with fitqtl to fit a defined multiple-QTL model (Broman *et al*. 2003). For the QTL analysis, a genome-wide significance level was calculated using 10 000 permutation tests with a significant ($\alpha = 0{\cdot}05$) LOD score of 3·90 and a suggestive ($\alpha = 0{\cdot}1$) LOD score of 3·46 after five weeks and a significant ($\alpha = 0{\cdot}05$) LOD score of 3·79 and a suggestive ($\alpha = 0{\cdot}1$) LOD score of 3·44 after 30 weeks. Since the Haley-Knott method can produce biased estimates of residual variance and can be sensitive to epistasis and linkage between QTL, estimating equations (Feenstra *et al*. 2006) were also used to conduct QTL mapping (equivalent to extended Haley–Knott regressions in Rqtl). The results of the extended Haley–Knott regression were the same as the above described Haley–Knott regression and are therefore not reported (but see Supplementary Methods). Both analyses are robust to the use of proportion data.

RESULTS

Persistence of H. tvaerminnensis

All controls were uninfected at each examination and will not be further discussed here.

After 5 weeks, all nine replicates of the Finnish parental clone (Xinb3) were infected, while only two of the nine replicates of the German parental clone (Iinb1) showed signs of infection (Fisher exact test: $P = 0{\cdot}0023$). The F_1 hybrid clone had six out of nine replicates infected. The majority of the 200 infected F_2 clones had all three replicates infected, with only seven clones showing no parasites in any replicate (Fig. 1a). In total, 79·5% of all replicates were infected. After 30 weeks, 49·5% of all replicates remained infected. Eight of the nine replicate populations from the Finnish parental clone (the susceptible parent clone of the F_2 panel) were still infected, but none of the nine replicate populations from the German parental clone (the resistant clone) were infected (Fisher exact test: $P = 0{\cdot}00041$). Three of the nine replicates of the F_1 clone remained infected. Among the 200 F_2 clones, three clones had one or two replicates die. These three clones are excluded from Fig. 1b. Around 25% of the 197 F_2 clones had none, one, two or three replicates infected (Fig. 1b).

QTL mapping

After 5 weeks, the single-QTL genome scan identified a QTL on linkage group (lg) 1 ($LOD_1 = 5{\cdot}37$, $LOD_{threshold} = 3{\cdot}90$; Fig. 1c, Tables 1 and 2) that explains 11·6% of the variation among the F_2 clones. One epistatic interaction between the same locus on lg 1 and another on lg 3 was detected (Tables 1 and 2), explaining 22·1% of the phenotypic variation.

After 30 weeks, a single QTL scan identified two QTL for persistence. The first one is located on lg 1 ($LOD_1 = 3{\cdot}89$, $LOD_{threshold} = 3{\cdot}79$) and explains 8·6% of phenotypic variation; the second one is on lg 6 ($LOD_6 = 4{\cdot}19$), explaining 9·2% variation (Fig. 1c). Together, the two QTL explain 16·8% variation. For both of the QTL, clones carrying the B (Finnish) genotype showed higher persistence rates than those with A (German) genotypes (Fig. 1d, e), which is in line with what we would expect from the parents. The QTL on lg 6 showed interaction with another QTL on lg 1 (Fig. 1f), explaining 19·5% variation. A model with all QTL and interaction terms explained 21·6% of the total phenotypic variation (Table 2). Another interaction between lg 1 and lg 8 was also detected, but since the locus on lg 8 is closely located to an infertility allele (red dwarf mutant, Routtu *et al*. 2014), there were only a few homozygotes for this locus (AA females at this region are sterile). Hence, this interaction could not be analysed in detail and will not be discussed further.

Comparison with early QTL study

Of the F_2 clones in our study, 129 were also used in an earlier study (Routtu and Ebert, 2015) that estimated spore production of *H. tvaerminnensis* after horizontal and vertical infections. The spore counts (clonal means) after horizontal and vertical infection of the *Daphnia* in that study correlated positively with the proportion of infected host populations in the current study at week 30 but not at week 5. Horizontal spore counts were (Spearman, $\rho = 0{\cdot}391$, $P < 0{\cdot}0001$) at week 30 and (Spearman, $\rho = -0{\cdot}005$, $P = 0{\cdot}95$) at week 5. Vertical infection spore counts were week 30: $\rho = 0{\cdot}255$, $P = 0{\cdot}0035$ and week 5: $\rho = 0{\cdot}07$, $P = 0{\cdot}42$. Thus, both vertical and horizontal spore counts are better predictors of long-term persistence than of short-term infection success.

In the current study, the QTL on lg 6 for persistence at week 30 (Q3, Table 1) is within the 95% confidence interval of a QTL for spore counts after vertical infections, as reported for the same QTL F_2 panel in the spore count study (Routtu and Ebert, 2015).

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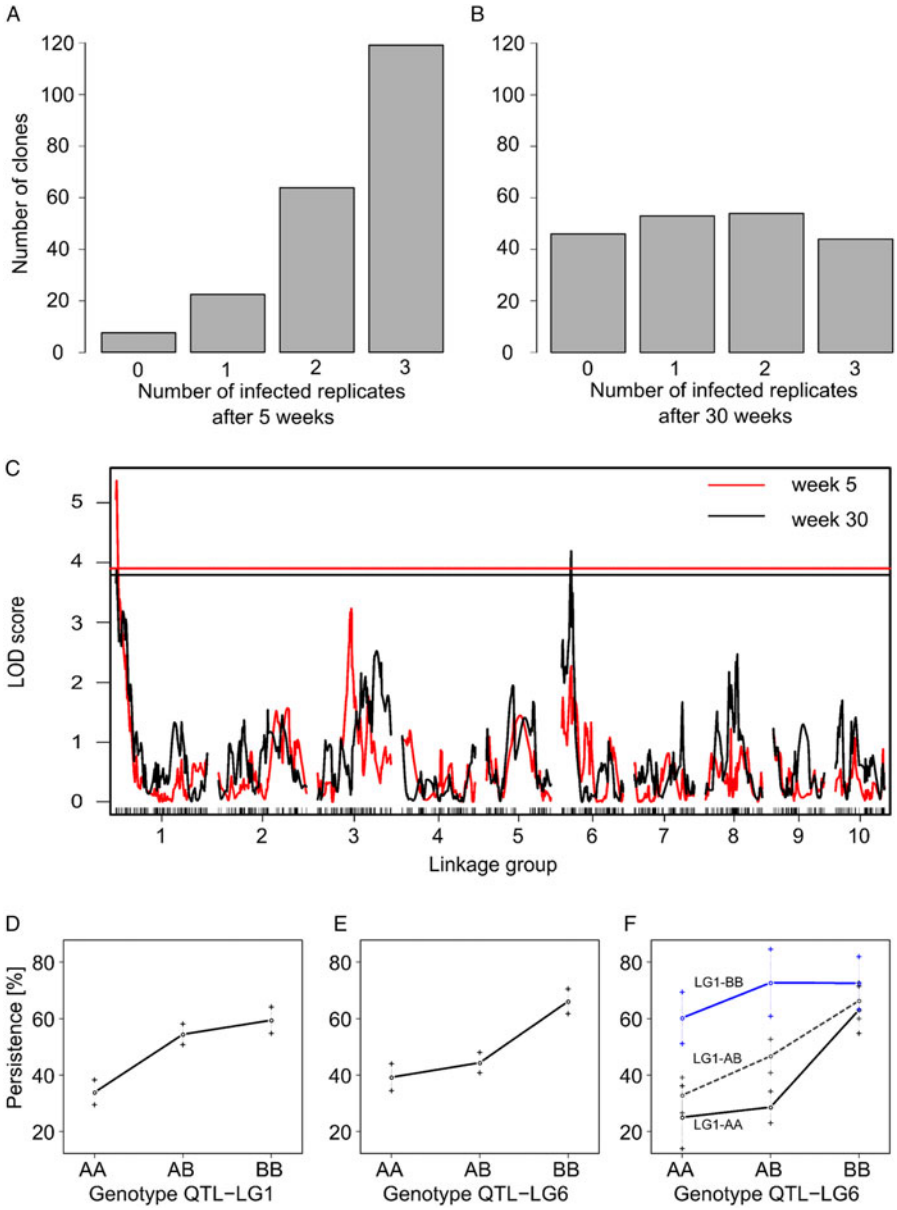

Fig. 1. Analysis of infection data after exposure to *H. tvaerminnensis*. (a) Frequency distribution of proportion data of the 200 F_2 clones showing the number of infected replicates (out of three) after 5 weeks. (b) As in (a) but for data from 197 F_2 clones after 30 weeks. (c) QTL mapping at 5 (in red) and 30 (in black) weeks persistence of *H. tvaerminnensis*. The horizontal lines indicate significance thresholds after 5 and 30 weeks. (d–f) Effect plots (±S.E.) for the 30-week QTL on lg 1 (Q1), lg 6 (Q3) and interaction lg 1–lg 6 (Q1 + Q3). Note that the QTL on lg 1 (Q1, position 2·35) was found in both weeks 5 and 30 (see Table 1).

None of the other QTL reported in the earlier study overlap with the QTL identified in this study.

Minor genetic effects

To test if other alleles from the susceptible Finnish parent clone contributed to susceptibility, we calculated the number of A and B alleles at all 1324 markers for each F_2 clone. We then regressed the proportion of infected replicates against the proportion of Finnish (B) alleles. The higher the proportion of Finnish alleles (B content), the higher the proportion of infected replicates ($P < 0{\cdot}001$, $r^2 = 0{\cdot}058$). This regression remained significant when all markers ±25 cM around the QTL listed in Table 1 were removed from the analysis ($P = 0{\cdot}0048$, $r^2 = 0{\cdot}0399$, Fig. 2). When the entire linkage groups lg1 and lg6 were removed from this analysis (with only 1035 markers remaining), the regression became non-significant ($P = 0{\cdot}39$, $r^2 = 0{\cdot}022$).

DISCUSSION

Long-term persistence is a complex, quantitative trait

This study sought to better understand the genetics underlying the long-term persistence of *H.*

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Table 1. Position of QTLs that contribute to persistence of *H. tvaerminnensis* with their corresponding marker names, positions of the SNP nucleotides (lowercase letters) and sequences on both sides of the SNP.

Trait	Linkage group	Position	Marker (SNP nucleotide)	Sequence (5′–3′)	QTL site
Persistence after 5 weeks	1	2·35	scaffold01302_1996	CCACAAGATTGAACAa GACTCTGCTACTGC	Q1
	3	60·90	scaffold02593_1287	CGTCAAAAaCATGAAA TaCGACAACTGTGGCG	Q2
Persistence after 30 weeks	1	2·35	scaffold01302_1996	CCACAAGATTGAACAa GACTCTGCTACTGC	Q1
	1	16·19	scaffold03191_677	GACACATGATCTAAATT TaCTGTCCAATGAAGC	Q2
	6	20·34	scaffold00815_3177	CGCAATAATAGAATGaG ATTTTTGTTCTATTTTCT	Q3

Position is given in centiMorgan of the respective linkage group. The marker sites and the SNP position refer to the *D. magna* genome (assembly 2·4). SNPs on scaffolds are numbered continuously.

Table 2. Phenotypic variation of parasite persistence explained by genotype effects at the QTL sites given in Table 1.

Trait	Model	D.F.	Mean square	LOD	% Variance explained
Persistence after 5 weeks	y ~ Q1	2	0·849	5·371	11·63
	y ~ Q1*Q2	8	0·403	10·826	22·06
Persistence after 30 weeks	y ~ Q1	2	1·091	3·892	8·57
	y ~ Q3	2	1·172	4·192	9·20
	y ~ Q1 + Q3	4	1·069	7·984	16·79
	y ~ Q2*Q3	8	0·620	9·410	19·48
	y ~ Q1 + Q2*Q3	10	0·551	10·578	21·62

All models are significant ($P < 0·0001$).

tvaerminnensis in *D. magna*. Our results suggest that the host genotype's ability to allow long-term parasite persistence is a quantitative trait, with multiple QTL strong enough to be picked up by our analysis. This finding coincides with Routtu and Ebert's (2015) spore count analysis conducted with the same parasite isolate in the same QTL F_2 panel. It offers a quantitative genetic picture for long-term persistence that contrasts with the bimodal pattern observed across natural genotypes – in which all replicates of *D. magna* genotypes from the parasite's native geographic range remain infected, while those outside its native range do not (Lange *et al*. 2015). Our results overall agree with the only other QTL study of microsporidian–host interactions we are aware of (Huang *et al*. 2014). In that study as well, resistance is a complex, quantitative trait with multiple QTL and epistatic interactions. The finding of multiple QTL for resistance against parasites is common (Behrens *et al*. 2011; Atlija *et al*. 2015; Henning *et al*. 2015), but not universal (Bento *et al*. 2017).

After 5 weeks, nearly 80% of all replicates in our experimental monoclonal *D. magna* populations were infected with *H. tvaerminnensis*, indicating that most F_2 clones are susceptible to the parasite after exposure to water-borne spores (horizontal transmission). However, after 30 weeks, only about half of the replicates remained infected. This marked decline in the frequency distribution of infected clone replicates between weeks 5 and 30 was also observed in the Lange *et al*. (2015) study using host genotypes from natural populations. These results underscore that, the parasite's ability to cause infection is not in actuality a good predictor of long-term persistence. Unlike the earlier study with the natural host genotypes, we found, after 30 weeks, that the numbers of infected replicates among the F_2 clones were more-or-less evenly distributed. The finding by Lange *et al*. (2015) that most clone replicates were either all infected or all uninfected after 25 weeks raised our expectations of finding a similar bimodal infection pattern among the F_2 clones. Such a pattern would have indicated a simple genetic mechanism with few genes and strong effects. This is clearly not the case, however; rather, a more complex genetic architecture underlies the observed phenotypic trait.

Our QTL analysis revealed two QTL for week 5 and three QTL for week 30, one of them (lg1, 2·35 cM) being the same. The QTL on lg 6 for long-term persistence (week 30) is within the 95% confidence interval of a QTL for spore counts after vertical infections, which was reported for animals

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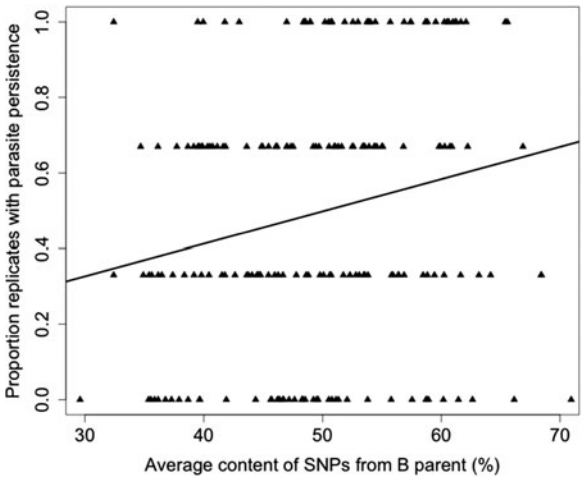

Fig. 2. Proportion of replicates with parasite persistence at week 30 plotted against the proportion of map markers in the F_2 clones derived from the Finnish parent clone (B genotype). Markers within ±25 cM around the QTL detected in this study were excluded. $N = 200$ F_2 clones. The black line is the regression line.

of the same QTL F_2 panel in an earlier study (Routtu and Ebert, 2015). This finding underlines the important role of vertical transmission for *H. tvaerminnensis* in *D. magna* populations (Lass and Ebert, 2006). Since only horizontal infections are possible in the initial phase of the experiment, it is not surprising that this QTL for vertical transmission did neither show up in our 6-week analysis for parasite presence, nor in the study on spore counts after horizontal infections (Routtu and Ebert, 2015).

Our results indicate that the *D. magna* host clone's ability to allow the parasite to persist is a quantitative genetic trait characterized by several loci, their interactions, and by environmental effects that contribute to trait expression. First, the QTL described here for parasite persistence at weeks 5 and 30 explains only about 22% of the phenotypic variation. For comparison, in another study using the same QTL panel, a single QTL for resistance against the bacterial parasite *Pasteuria ramosa*, explained 50% of the phenotypic variation (Routtu and Ebert, 2015). Second, a regression of persistence against the number of B alleles (the alleles of the susceptible Finnish parent genotype) showed a positive slope, even after the regions around the QTL were removed. This finding suggests that other loci with small effects may accumulate to further influence variance. Third, we observed that in about half of the F_2 clones, one or two replicates out of three were still infected after 30 weeks. Persistence is a threshold trait and is not possible when $R_0 < 1$ (Woolhouse and Antia, 2008). When the mean R_0 of the parasite in a population of a F_2 clone is around 1, microenvironmental factors may cause some replicates to be below 1 and others to be above. In these cases, stochastic effects will contribute to trait expression, as is typical for quantitative traits (Falconer and MacKay, 1996). Our earlier study on natural *D. magna* genotypes showed a clear bimodal distribution across replicates of the host genotypes, suggesting that R_0 in these monoclonal populations was either clearly below or above one (Lange *et al.* 2015), thus reducing the consequences of stochastic effects on long-term persistence. As both studies were conducted in the same laboratory under the same conditions, it seems unlikely that this difference is due to an unexplained experiment-wise effect. Rather, the crossing of resistant and susceptible parent genotypes yielded F_2 genotypes with mostly intermediate genotypic means, as is expected for quantitative traits (Falconer and MacKay, 1996).

Our study is based on a QTL cross resulting from two parent lines with divergent phenotypes and one single F_1 one hybrid. The variation we observed results from the segregation and recombination of alleles present in this F_1 genotype, which was selfed to produce the F_2 panel. Therefore, the genetic variation uncovered is likely only a subset of what is present in natural population. Given however, that the two parent clones come from geographic regions where relatively low levels of genetic variation for *Daphnia–Hamiltosporidium* interactions have been observed (Ebert, 2008; Lange *et al.* 2015), we believe that our F_2 panel captures much of the variation that characterizes the divergence among these regions. However, to verify this, further crosses would be necessary.

Long-term persistence correlate with host habitat

Lange *et al.* (2015) observed that *D. magna* genotypes from ponds that are likely to dry up in the summer allow for parasite persistence. This includes all ponds from the Finnish and Swedish coasts (shallow rock pools) and in the Mediterranean region (France, Italy and Israel). It has been speculated that it is *D. magna*'s adaptation to produce resting stages to survive the summer droughts in these ponds that make these *Daphnia*, at the same time, susceptible to long-term *H. tvaerminnensis* persistence (Lange *et al.* 2015). This genetic trade-off between parasite resistance and drought tolerance would be an unusual example of a pleiotropic effect. Pleiotropic effects for parasite resistance and other fitness-related traits may be a general signature of resistance [reviewed by Schmid-Hempel (2011)] and have been observed in other systems. For example, resistance in *Drosophila* to a parasitoid wasp and to a bacterial parasite has fitness costs in the absence of the parasites (Kraaijeveld and Godfray, 1997; McKean *et al.* 2008). A cost of resistance has also been described for the *D. magna–H. tvaerminnensis* system (Zbinden *et al.* 2008). This study found that experimental evolution in the presence and absence of the parasite resulted in hosts being more and less tolerant to the parasite, but

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growing more slowly and more quickly (asexual growth rate), respectively. It is unclear, however, if this result is related to the long-term persistence variation discussed here.

Divergent selection between summer-dry and summer-wet habitats might have led *D. magna* populations to deviate in their ability to allow parasite persistence, creating the bimodal pattern observed across Europe (Lange *et al*. 2015). A link between habitat and parasite persistence may be the production of resting stages. Populations in habitats with a high propensity to dry up in summer must produce resting stages to survive this drought, which may be different from adaptations for surviving winter freezing. Unfortunately, nothing is known about differences in resting stages between summer dry and summer wet *D. magna* populations. In a QTL study about resting egg production, which also seems to be a quantitative trait, there was a genetic interaction with a locus on lg 6 (Roulin *et al*. 2013), which falls within the 95% CI (confidence interval) range of the QTL for parasite persistence on lg 6. However, in our 30-week population experiment, resting stages were not a factor, as all populations were kept under asexual growth conditions. Even though many of the F_2 clones did produce resting stages during the experiment, these sank to the bottom of the jars and did not hatch.

Hamiltosporidium tvaerminnensis has a considerable negative effect on the fecundity, life span and competitive ability of *D. magna* and may reach local prevalence of up to 100%. In the parasite's native geographic range, many host populations harbour the parasite (Ebert *et al*. 2001; Goren and Ben-Ami, 2013), in contrast to central Europe, where the parasite has never been observed, even though *D. magna* is abundant. Given the host's strong tendency and proficiency for migration, some constraint must exist that prevents the spread of resistant host genotypes into *D. magna* populations in the parasite's geographic range. Likewise, since the parasite is very efficiently transmitted through the resting and dispersal stages of the host (Ebert *et al*. 2007), one would assume that *H. tvaerminnensis* is repeatedly introduced into areas outside its current range. However, host resistance in these populations seems to prevent the establishment of the parasite there. Thus, the resistance of *D. magna* against *H. tvaerminnensis*, possibly linked with the inability of these hosts to survive other than as ephippia in summer-dry habitats, seems to explain the geographic host range of the parasite in the much more widely distributed host species.

Concluding remarks

The aim of this study was to elucidate the genetic architecture underlying the ability of *D. magna* host clones to allow for the long-term persistence of a microsproridian parasite. We found that long-term persistence shows a phenotypic distribution typical of a complex, quantitative genetic trait. This quantitative pattern is substantiated by the genetic architecture our study uncovered, which suggests few major QTL, epistasis among QTL, and the presence of minor QTL. This is consistent with an earlier study that reported that the spore count of this parasite across hosts from the same QTL F_2 panel is also a quantitative trait. These findings are surprising because the parasite has a disjunct geographic distribution, and hosts from regions where the parasite is absent are resistant to it, whereas hosts in the centre of the parasite's range are susceptible (Ebert, 2008; Lange *et al*. 2015). We speculate that local adaptation by hosts drove genetic divergence at various loci in the genome, resulting in extreme phenotypes. Alleles at these loci, segregating and recombining in the F_2 lines of the QTL cross, produced the quantitative picture observed here. It is currently not know what selective agent maintains the bimodal distribution of quantitative resistance in this host–parasite system.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182017001032.

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REFERENCES

Agampodi, S. B. and Wickramage, K. (2013). Is there a risk of yellow fever virus transmission in South Asian countries with hyperendemic dengue? *BioMed Research International* **2013**, 905043.

Antonovics, J., Boots, M., Ebert, D., Koskella, B., Poss, M. and Sadd, B. M. (2013). The origin of specificity by means of natural selection: evolved and nonhost resistance in host–pathogen interactions. *Evolution* **67**, 1–9.

Atlija, M., Arranz, J. J., Martinez-Valladares, M. and Gutierrez-Gil, B. (2015). Detection and replication of QTL underlying resistance to gastrointestinal nematodes in adult sheep using the ovine 50 K SNP array. *Genetics Selection Evolution* **48**, 4.

Behrens, D., Huang, Q., Gessner, C., Rosenkranz, P., Frey, E., Locke, B., Moritz, R. F. A. and Kraus, F. B. (2011). Three QTL in the honey bee *Apis mellifera* L. suppress reproduction of the parasitic mite *Varroa destructor*. *Ecology and Evolution* **1**, 451–458.

Ben-Ami, F., Mouton, L. and Ebert, D. (2008). The effects of multiple infections on the expression and evolution of virulence in a *Daphnia*–endoparasite system. *Evolution* **62**, 1700–1711.

Bento, G., Routtu, J., Fields, P. D., Bourgeois, Y., Du Pasquier, L. and Ebert, D. (2017). The genetic basis of resistance and matching-allele interactions of a host–parasite system: the *Daphnia magna–Pasteuria ramosa* model. *PLoS Genetics* **13**, e1006596.

Bieger, A. and Ebert, D. (2009). Expression of parasite virulence at different host population densities under natural conditions. *Oecologia* (*Berlin*) **160**, 247–255.
Broman, K. W. and Sen, Ś. (2009). *A Guide to QTL Mapping with R/qtl*. Springer, New York.
Broman, K. W., Wu, H., Sen, S. and Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**, 889–890.
Combes, C. (2001). *Parasitism: the Ecology and Evolution of Intimate Interactions*. 552 pp. University of Chicago Press.
Corradi, N., Haag, K. L., Pombert, J. F., Ebert, D. and Keeling, P. J. (2009). Draft genome sequence of the *Daphnia* pathogen *Octosporea bayeri:* insights into the gene content of a large microsporidian genome and a model for host–parasite interactions. *Genome Biology* **10**, R106.
Culleton, R. L., Mita, T., Ndounga, M., Unger, H., Cravo, P. V. L., Paganotti, G. M., Takahashi, N., Kaneko, A., Eto, H., Tinto, H., Karema, C., D'Alessandro, U., do Rosário, V., Kobayakawa, T., Ntoumi, F., Carter, R. and Tanabe, K. (2008). Failure to detect *Plasmodium vivax* in West and Central Africa by PCR species typing. *Malaria Journal* **7**, 174.
De Gelas, K. and De Meester, L. (2005). Phylogeography of *Daphnia magna* in Europe. *Molecular Ecology* **14**, 753–764.
Dobson, A., Molnar, P. K. and Kutz, S. (2015). Climate change and Arctic parasites. *Trends in Parasitology* **31**, 181–188.
Ebert, D. (2008). Host–parasite coevolution: insights from the Daphnia–parasite model system. *Current Opinion in Microbiology* **11**, 290–301.
Ebert, D. (2013). The epidemiology and evolution of symbionts with mixed-mode transmission. *Annual Review of Ecology, Evolution, and Systematics* **44**, 623–643.
Ebert, D., Hottinger, J. W. and Pajunen, V. I. (2001). Temporal and spatial dynamics of parasites in a *Daphnia* metapopulation: which factors explain parasite richness? *Ecology* **82**, 3417–3434.
Ebert, D., Altermatt, F. and Lass, S. (2007). A short term benefit for outcrossing in a *Daphnia* metapopulation in relation to parasitism. *Journal of the Royal Society Interface* **4**, 777–785.
Falconer, D. S. and MacKay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th Edn. Longman, Harlow, UK.
Feenstra, B., Skovgaard, L. A. and Broman, K. W. (2006). Mapping quantitative trait loci by an extension of the Haley–Knott regression method using estimating equations. *Genetics* **173**, 2269–2282.
Fields, P. D., Reisser, C., Dukic, M., Haag, C. R. and Ebert, D. (2015). Genes mirror geography in *Daphnia magna*. *Molecular Ecology* **24**, 4521–4536.
Gagne, R. B., Hogan, J. D., Pracheil, B. M., Mcintyre, P. B., Hain, E. F., Gilliam, J. F. and Blum, M. J. (2015). Spread of an introduced parasite across the Hawaiian archipelago independent of its introduced host. *Freshwater Biology* **60**, 311–322.
Goren, L. and Ben-Ami, F. (2013). Ecological correlates between cladocerans and their endoparasites from permanent and rain pools: patterns in community composition and diversity. *Hydrobiologia* **701**, 13–23.
Guerra, C. a., Howes, R. E., Patil, A. P., Gething, P. W., Van Boeckel, T. P., Temperley, W. H., Kabaria, C. W., Tatem, A. J., Manh, B. H., Elyazar, I. R. F., Baird, J. K., Snow, R. W. and Hay, S. I. (2010). The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *PLoS Neglected Tropical Diseases* **4**, e774.
Haag, K. L., Larsson, J. I. R., Refardt, D. and Ebert, D. (2011). Cytological and molecular description of *Hamiltosporidium tvaerminnensis* gen. et sp nov., a microsporidian parasite of *Daphnia magna*, and establishment of *Hamiltosporidium magnivora* comb. nov. *Parasitology* **138**, 447–462.
Haag, K. L., Sheikh-Jabbari, E., Ben-Ami, F. and Ebert, D. (2013*a*). Microsatellite and single-nucleotide polymorphisms indicate recurrent transitions to asexuality in a microsporidian parasite. *Journal of evolutionary Biology* **26**, 1117–1128.
Haag, K. L., Traunecker, E. and Ebert, D. (2013*b*). Single-nucleotide polymorphisms of two closely related microsporidian parasites suggest a clonal population expansion after the last glaciation. *Molecular Ecology* **22**, 314–326.
Haley, C. S. and Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315–324.
Henning, J. A., Gent, D. H., Twomey, M. C., Townsend, M. S., Pitra, N. J. and Matthews, P. D. (2015). Precision QTL mapping of downy mildew resistance in hop (*Humulus lupulus* L.). *Euphytica* **202**, 487–498.
Huang, Q., Kryger, P., Le Conte, Y., Lattorff, H. M. G., Kraus, F. B. and Moritz, R. F. A. (2014). Four quantitative trait loci associated with low *Nosema ceranae* (Microsporidia) spore load in the honeybee *Apis mellifera*. *Apidologie* **45**, 248–256.
Huyse, T., Poulin, R. and Theron, A. (2005). Speciation in parasites: a population genetics approach. *Trends in Parasitology* **21**, 469–475.
Klüttgen, B., Dülmer, U., Engels, M. and Ratte, H. T. (1994). ADaM, an artificial freshwater for the culture of zooplankton. *Water Research* **28**, 743–746.
Kraaijeveld, A. R. and Godfray, H. C. J. (1997). Tradeoff between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**, 278–280.
Lange, B., Kaufmann, A. P. and Ebert, D. (2015). Genetic, ecological and geographic covariables explaining host range and specificity of a microsporidian parasite. *Journal of Animal Ecology* **84**, 1711–1719.
Lass, S. and Ebert, D. (2006). Apparent seasonality of parasite dynamics: analysis of cyclic prevalence patterns. *Proceedings of the Royal Society of London B: Biological Sciences* **273**, 199–206.
Lass, S., Hottinger, J. W., Fabbro, T. and Ebert, D. (2011). Converging seasonal prevalence dynamics in experimental epidemics. *BMC Ecology* **11**, 14.
McKean, K. a., Yourth, C. P., Lazzaro, B. P. and Clark, A. G. (2008). The evolutionary costs of immunological maintenance and deployment. *BMC Evolutionary Biology* **8**, 76.
Poulin, R. (2007). *Evolutionary Ecology of Parasites*. Princeton University Press, Princeton, USA.
Poulin, R. and Keeney, D. B. (2008). Host specificity under molecular and experimental scrutiny. *Trends in Parasitology* **24**, 24–28.
R Development Core Team (2008). *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.
Refardt, D. and Ebert, D. (2007). Inference of parasite local adaptation using two different fitness components. *Journal of evolutionary Biology* **20**, 921–929.
Rogers, D. J., Wilson, A. J., Hay, S. I. and Graham, A. J. (2006). Global mapping of infectious diseases: methods, examples and emerging applications. *Advances in Parasitology* **62**, 181–220.
Roulin, A. C., Routtu, J., Hall, M. D., Janicke, T., Colson, I., Haag, C. R. and Ebert, D. (2013). Local adaptation of sex induction in a facultative sexual crustacean: insights from QTL mapping and natural populations of *Daphnia magna*. *Molecular Ecology* **22**, 3567–3579.
Roulin, A. C., Bourgeois, Y., Stiefel, U., Walser, J. C. and Ebert, D. (2016). A photoreceptor contributes to the natural variation of diapause induction in *Daphnia magna*. *Molecular Biology and Evolution* **33**, 3194–3204.
Routtu, J. and Ebert, D. (2015). Genetic architecture of resistance in *Daphnia* hosts against two species of host-specific parasites. *Heredity* **114**, 241–248.
Routtu, J., Jansen, B., Colson, I., De Meester, L. and Ebert, D. (2010). The first-generation *Daphnia magna* linkage map. *Bmc Genomics* **11**, 508.
Routtu, J., Hall, M. D., Albere, B., Beisel, C., Bergeron, R. D., Chaturvedi, A., Choi, J. H., Colbourne, J., De Meester, L., Stephens, M. T., Stelzer, C. P., Solorzano, E., Thomas, W. K., Pfrender, M. E. and Ebert, D. (2014). An SNP-based second-generation genetic map of *Daphnia magna* and its application to QTL analysis of phenotypic traits. *BMC Genomics* **15**, 1033.
Ryan, S. J., McNally, A., Johnson, L. R., Mordecai, E. A., Ben-Horin, T., Paaijmans, K. and Lafferty, K. D. (2015). Mapping physiological suitability limits for malaria in Africa under climate change. *Vector-Borne and Zoonotic Diseases* **15**, 718–725.
Schmid-Hempel, P. (2011). *Evolutionary Parasitology*. Oxford University Press, Oxford, UK.
Vizoso, D. B. and Ebert, D. (2005). Mixed inoculations of a microsporidian parasite with horizontal and vertical infections. *Oecologia* (*Berlin*) **143**, 157–166.
Vizoso, D. B., Lass, S. and Ebert, D. (2005). Different mechanisms of transmission of the microsporidium *Octosporea bayeri*: a cocktail of solutions for the problem of parasite permanence. *Parasitology* **130**, 501–509.
Wolfe, N. D., Switzer, W. M., Carr, J. K., Bhullar, V. B., Shanmugam, V., Tamoufe, U., Prosser, A. T., Torimiro, J. N., Wright, A., Mpoudi-ngole, E., Mccutchan, F. E., Birx, D. L., Folks, T. M., Burke, D. S. and Heneine, W. (2004). Naturally acquired simian retrovirus infections in central African hunters. *The Lancet* **363**, 932–937.
Woolhouse, M. E. and Antia, R. (2008). Emergence of new diseases. In *Evolution in Health and Disease*, 2nd Edn (ed. Stearns, S. C. and Koella, J. K.), pp. 215–228. Oxford University Press, Oxford, UK.
Zbinden, M., Haag, C. R. and Ebert, D. (2008). Experimental evolution of field populations of *Daphnia magna* in response to parasite treatment. *Journal of Evolutionary Biology* **21**, 1088–1078.