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Genetic, ecological and geographic covariables explaining host range and specificity of a microsporidian parasite

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Summary

1. Parasites often have a smaller geographic distribution than their hosts. Common garden infection trials can untangle the role that historical contingencies, ecological conditions and the genetic constitution of local host populations play in limiting parasite geographic range; however, infection trials usually overestimate the range of hosts in which a parasite could naturally persist.

2. This study overcomes that problem by using multigeneration, long-term persistence experiments. We study the microsporidian parasite *Hamiltosporidium tvaerminnensis* in monoclonal populations of *Daphnia magna* from 43 widely spread sites. The parasite persisted well in hosts collected from its natural geographic range, but demonstrated long-term persistence in only a few host genotypes outside this range.

3. Genetic distance between hosts from the parasite's origin site and newly tested host populations correlated negatively with parasite persistence. Furthermore, the parasite persisted only in host populations from habitats with a high likelihood of drying up in summer, although we excluded environmental variation in our experiments.

4. Together, our results suggest that host genetic factors play the dominant role in explaining the limited geographic range of parasites and that these genetic differences covary with geographic distance and the habitat type the host is adapted to.

Key-words: host–parasite, pathogen, populations, resistance, transmission

Introduction

Most parasites can only infect and reproduce in a limited range of host genotypes or species. Understanding the factors that determine these parasite host ranges can yield greater understanding of the evolutionary ecology of parasites. A broad host range can benefit a parasite by providing a large array of potential resources (Poulin 2007; Koehler *et al.* 2012) and better chances of survival (Clavel, Julliard & Devictor 2011). However, parasites may be driven to specialization, exhibiting adaptation to the local ecological conditions or to the local host population, evolving greater success in host exploitation and improved intraniche competition (Tripet, Christe & Møller 2002; Greischar & Koskella 2007; Arbiv *et al.* 2012). This specialization comes at a cost for the parasite, however, in having its evolutionary fate more tightly connected to its host's, thus increasing the local risk of parasite extinction. Although host ranges are important for the evolutionary ecology of parasites, little is known about the factors that determine these ranges, nor how to accurately assess them. Not surprisingly, the evolution of host ranges has become an important topic in ecological and evolutionary research (for reviews, see Poulin 2007; Schmid-Hempel 2011; Antonovics *et al.* 2013).

Host range studies have frequently stressed the importance of ecological and genetic similarity between the host population (source or native) from which the parasite was collected and the host population on which it was tested. For example, studies have shown that geographic distance (as a proxy for genetic similarity) (Ebert 1994; Morand, Manning & Woolhouse 1996), genetic distance (Perlman & Jaenike 2003; Longdon *et al.* 2011) and ecological similarity (Becerra & Venable 1999) correlate with the

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likelihood that a parasite is able to infect a host outside its native range (see Poulin 2007; Schmid-Hempel 2011; Antonovics *et al*. 2013 for reviews). The influence of these predictors can be disentangled in part using common garden infection experiments that expose hosts from different locations or species to the parasite and quantify infection success. Interestingly, however, experimental host range studies often result in a broader host range than is observed in nature (Futuyma, Keese & Funk 1995; Moore & Gotelli 1996; Reed & Hafner 1997; Mutikainen *et al*. 2000; Morehead & Feener 2000; Perlman & Jaenike 2003), making it difficult to relate experimentally determined host ranges to observations from the field. Several scenarios may be behind this discrepancy: first, the natural host range may be limited by parasite dispersal, making host populations outside the parasite's range naturally parasite free. Alternatively, the experimental host range may have been tested in ecological conditions suitable for the parasite, although these conditions may not be present in all natural populations of potential hosts. Another hypothesis is that the host range experiment did not include the parasite's entire life cycle. If infection trials do not assess the parasite's ability to transmit to further host individuals, it is impossible to definitively determine the parasite's ability to persist in host populations (Woolhouse & Gowtage-Sequeria 2005). A recent study of a bacterial pathogen, for example, showed that the parasite's host range was limited by the exclusion of only one step needed for its growth cycle (Luijckx *et al*. 2013). Another study found that the intensity of the parasite infection did not reliably predict parasite persistence in experimental host populations, presumably because high infection intensity did not lead to high transmission success (Refardt & Ebert 2007). A further investigation showed that parasite fitness can be determined by diet-quality-dependent interspecific parasite interactions, when host population-level effects are taken into account (Lange *et al*. 2014). This suggests that experimental host range studies should include the parasite's entire transmission cycle to better assess its ability for long-term persistence in a host population.

To better understand the factors that determine the host range of parasites, we studied the microsporidian parasite *Hamiltosporidium tvaerminnensis* (Haag *et al*. 2011), which has been reported only in *Daphnia magna* rock pool populations along the eastern Swedish and southern Finnish coast of the Baltic Sea (Ebert 2005; Haag, Traunecker & Ebert 2013; Haag *et al*. 2013), although its host species is commonly found throughout Eurasia and Africa. A dispersal barrier limiting this parasite to a small geographic region seems unlikely, since the parasite can survive and co-migrate in the resting eggs of its host (Vizoso, Lass & Ebert 2005), and *D. magna* population genetics support high migration rates among European populations (De Gelas & De Meester 2005). In this study, we tested *H. tvaerminnensis* parasite performance in host populations across Eurasia using short- and long-term persistence assays in experimental monoclonal host populations. Long-term persistence assays included several complete parasite transmission cycles and tested three explanatory variables for host range: genetic distance between source host and new host, geographic distance between source host and new host, and habitat type of the hosts. We found that long-term persistence resulted in the most narrow host range and corresponded well, but not perfectly, with the natural distribution of the parasite. All three explanatory variables revealed significant correlations with host range. Since all experimental populations were kept under the same conditions, the finding of a correlation between the host's native habitat and parasite persistence suggests that host adaptation to summer-dry habitats may facilitate parasite persistence.

Materials and methods

DAPHNIA CLONES

Daphnia magna Straus 1820 is a cyclic parthenogenetic planktonic crustacean that inhabits freshwater ponds and lakes. Offspring are produced from asexual eggs during advantageous environmental conditions and from resting eggs (=ephippia) when environmental conditions deteriorate (Kleiven, Larsson & Hoebæk 1992). Resting eggs are sexually produced and allow *Daphnia* to outlast adverse environmental conditions, such as freezing or drying. Monoclonal populations can be obtained by keeping females singly under good conditions, allowing for asexual reproduction. In this study, clones from 43 *Daphnia magna* populations originating from sites across Eurasia and Kenya were used (Table 1). Clones infected with vertically transmitted microsporidian parasites were cured in the laboratory before the experiments began (Zbinden *et al*. 2005). Following Roulin *et al*. (2013), we classified the water habitat of all populations into one of four categories: permanent water year-round (mostly coastal western Europe), winter freezing (mostly northern and north-eastern populations), summer dry (Mediterranean populations) and winter freezing plus high likelihood of summer dry, (rock pools in northern Europe), and the later was termed 'ephemeral' in the study by Roulin *et al*. (2013). Summer dryness was determined based on observations by ourself or by observations by people providing the *Daphnia* samples. For Scandinavian pools, we further used a desiccation model (Altermatt, Pajunen & Ebert 2009). Winter freezing was estimated based on the average temperature of the coldest month. If the average temperature in the coldest month was below 0 °C, we assumed the water body would freeze over in winter. Single clones from five other *Daphnia* species were also included: *D. similis* from Israel, *D. dolichocephala* from South Africa, *D. lumholtzi* from Zimbabwe, *D. galeata* from Germany and *D. pulex* from Germany.

GENETIC ANALYSIS OF *DAPHNIA MAGNA* CLONES

We haplotyped all *D. magna* genotypes used in the experiments with the mitochondrial cytochrome c oxidase subunit I gene *(COI)*. DNA from two fresh animals was extracted using the 'DNeasy Blood and Tissue Kit' (Qiagen, Hilden, Germany). Universal arthropod *COI* primers (Folmer *et al*. 1994) were used to amplify the target gene *COI*. PCR was performed in a volume

Table 1. GLM results for the proportion of infected *Daphnia magna* populations after 5 and 25 weeks in dependence of (A) geographic distance (between parasite isolate and tested host genotype) and environmental conditions (four habitat types) and (B) the genetic distance between the host population from where the parasite isolate was collected from and host genotype in which the parasite was tested in. *Daphnia* genotypes that carried no infection in all experimental replicate populations at week 5 were excluded from the experiment at that point, reducing the sample size accordingly. All replicates from one clone from the White Sea (Russia) were lost before week 25. Since geographic and genetic distances are strongly confounded, it was not meaningful to include both variables in the same GLM. Changing the tested host clones between experiments to increase the reliability of our results led to block effects of experiment

Week	Variables	df	Residual deviance	Residual df	*F*	*P*
(A)						
5	Geographic distance	1	2917·3	64	209·72	<0·0001
	Environment	3	888·4	61	112·62	<0·0001
	Geographic distance * Environment	3	851·0	58	2·08	0·11
	Block (Experiment)	3	347·4	55	27·96	<0·0001
25	Geographic distance	1	1913·3	52	8534·69	<0·0001
	Environment	3	107·7	49	1805·58	<0·0001
	Geographic distance * Environment	3	10·8	46	96·84	<0·0001
	Block (Experiment)	3	8·6	43	3·28	<0·03
(B)						
5	Genetic distance	1	3380·6	66	931·35	<0·0001
	Block (Experiment)	1	1747·7	65	1632·90	<0·0001
25	Genetic distance	1	2899·6	53	982·77	<0·0001
	Block (Experiment)	1	2305·1	52	594·48	<0·0002

of 50 μL, consisting of 50 ng of template DNA, 20 pmol of each primer, 200 μM dNTPs, 1 U *Taq* DNA polymerase (Sigma, Munich, Germany), 10 mM Tris-HCL pH 8·3, 50 mM KCL, 1·5 mM $MgCL_2$ and 0·001% gelatin. A 'touchdown' PCR procedure was performed with an initial 3 min. step of denaturation at 94 °C, followed by 10 cycles of denaturation for 30 s at 94 °C, an annealing time of 30 s (in which the annealing temperature was decreased 1 °C every cycle, starting from 50 °C), and an extension step for 1 min. at 72 °C. These cycles were followed by 30 more cycles of annealing at 40 °C and a final extension at 72 °C for 6 min. (Haag, Traunecker & Ebert 2013). PCR products were purified with 'PCR purification kit' (Qiagen) and then sequenced in both directions (Microsynth, Balgach, Switzerland). Sequences were aligned with CodonCode Aligner (v. 3.7.1; CodonCode Corporation, Dedham, MA). Haplotype diversity of the sequences was calculated using the program DnaSP v5 (Librado & Rozas 2009), and the sequences were then used to compute a distance matrix (*Kimura* 2-parameter distance) with the program mega 5 (Tamura *et al.* 2011).

THE PARASITE

Hamiltosporidium tvaerminnensis Haag *et al.* (2011), formerly called *Octosporea bayeri*, is a horizontally and vertically transmitting microsporidian parasite. It transmits vertically to 100% of *D. magna's* asexual offspring, but to less than 100% of sexual offspring (Vizoso, Lass & Ebert 2005). Horizontal transmission occurs after the host's death, when spores of the decaying host cadaver are suspended in the water. Because of its combined vertical and horizontal transmission strategies, the parasite reaches 100% prevalence in asexual populations in both the field and the laboratory (Lass & Ebert 2006). Using infected female hosts collected from natural populations, the parasite can be cultivated in the laboratory for long periods of time. Previous genetic studies have shown very little sequence variation among the parasite's entire geographic range, suggesting that it may have originated from a single founder and subsequently reproduced exclusively asexually (Haag, Traunecker & Ebert 2013; Haag *et al.* 2013). In this study, we used three isolates of *H. tvaerminnensis* collected from the westernmost (Swedish coast), central (south-western Finish coast) and easternmost (Finnish–Russian border of the Baltic Sea) parts of its known geographic range. Phenotypic (spore morphology) and genetic analysis of these three isolates (Haag, Traunecker & Ebert 2013) revealed them to be nearly identical. Haag, Traunecker & Ebert (2013) suggested they are derived from the same *H. tvaerminnensis* clone that likely colonized the Baltic Sea region after the last ice age, less than 10 000 years ago.

We produced parasite spore suspensions for the infection experiments by crushing infected hosts in *Daphnia* medium. Spore concentrations were determined using a Thoma cell-counting chamber. Control suspensions were produced from uninfected hosts of the same host genotype.

EXPERIMENTS

We conducted three experiments, each following the same design with minor variations, and each using a different parasite isolate. Some host clones were used in all the experiments, while others were used only in single experiments (See Table S1, Supporting information). Experiment 1 contained 15 monoclonal populations (three controls + 6 replicates receiving a high parasite spore dose + 6 replicates with low spore dose) of each of 24 *D. magna* genotypes. Parasite spores were isolated from *D. magna* genotype FI-OER-3-3 from south-western Finland (59°49′N, 23°12′E). For each host genotype, six populations were exposed to 1200 spores mL^{-1} (low dose), six to 12 000 spores mL^{-1} (high dose) and three to controls. Since dose did not affect parasite persistence, experiments 2 and 3 applied only a high-spore dose to six replicate populations and a control suspension to three populations of each host genotype. To enlarge the number of experimentally tested host clones and ensure the consistency of experimental results, experiment 2 used 28 *D. magna* genotypes and parasite spores isolated from *D. magna* clone SE-G3-7 from

eastern Sweden (60°25′23″N, 18°33′26″E), whereas experiment 3 used 17 *D. magna* genotypes and parasite spores isolated from *D. magna* clone FI-FUT3-7-3 from eastern Finland (60°20′59″N, 27°26′34″E). Experiments 1 and 2 also included one clone each of five other *Daphnia* species: *D. dolichocephala, D. similis, D. pulex, D. galeata* and *D. lumholtzi*.

Daphnia populations were kept in 400-mL jars filled with 350 mL artificial freshwater medium (ADaM) (Klüttgen *et al.* 1994) (modified by using only 5% of the recommended SeO_2 concentration). Populations were fed 150 million cells (50 million cells for the smaller *D. galeata* and *D. lumholtzi*) of the chemostat grown green algae *Scenedesmus sp*. three times per week. Experimental populations were halved to generate replicate populations using a Folsom Plankton Divider and kept for 4 weeks before exposure to the parasites. All cultures and experiments were kept in a light: dark cycle of 16:8 h at 20 °C.

Treatments were started by adding spore suspension or control suspension to the population jars. All populations were divided into half every 5 weeks with the Folsom Plankton Divider. One half was used for data collection, while the other half stayed in the experiment. Although this procedure reduced population size by approximately 50% every 5 weeks, population size was quickly compensated by asexual reproduction of the *Daphnia*. The animals that remained in the experiment were transferred to fresh medium in clean jars. Before dividing, population sizes ranged from around 20 to 80 individuals per population. Population extinction was rare (<3%, see below). All host populations were checked for infection after 5 and 25 weeks by taking a sample of five large females from every host population and crushing it on a microscopic slide. *H. tvaerminnensis* was easily detected under phase contrast light microscope (400× magnification). If at least one host individual was infected, the replicate population was considered to support parasite persistence. In cases where populations showed infection at week 5, but not at the experiment's end, we examined ten additional host individuals for infection; however, in no case did this change the outcome. Host genotypes that did not show at least one infected replicate population at week 5 were removed from the experiment, assuming that the *Daphnia* genotype did not support parasite persistence. By week 25, none of the *Daphnia* individuals used to start the experiment were still alive, given that the maximum life span is about 100 days. Since the generation time of *D. magna* is 10–14 days, we assume that our experiment included at least 10 host generations. In experiment 2, an additional prevalence assessment was conducted after 5 weeks ($n = 24$ per replicate population).

STATISTICAL ANALYSIS

Because repeatedly tested host clones showed exactly the same parasite persistence in the three experiments (Tables S2–S4, Supporting information), we analysed and have presented the data of the three experiments together, with combinations being the unit of replication. We observed a block effect of experiment, since we changed the pool of tested host clones for each experiment to enlarge the number of tested host clones.

We calculated three types of data: parasite persistence as the proportion of infected populations for week 5 and week 25, geographic distance between the site of parasite origin and the site of host origin (0 if both are from the same population) and, finally, the genetic distance between the source host (clone from the population the parasite was isolated from) and the tested host clone (calculated from the genetic distance matrix).

We used a general linear model (GLM) of the quadratic-transformed infection proportion data (with quasibinomial error distribution) to analyse parasite persistence with the R software package (v 2.10.0; http://www.r-project.org). We ran the model first with geographic distance between host clone origin and parasite isolate origin (log_{10}-transformed) and habitat category as explanatory variables, and then, in a second analysis, with genetic distance between tested host clone and host clone from which the parasite was isolated as explanatory variables. Since geographic and genetic distances positively correlated, we did not include them in the same analysis to avoid the problem of collinearity. The unexposed control populations were not included in this analysis. For experiment 1, we also tested the effect of parasite spore dose using a Welch's two sample *t*-test to see whether the parasite affected the likelihood of host population extinction.

Results

GENETIC ANALYSIS

The 612-base pair fragment of the COI gene from all *D. magna* genotypes was sequenced and could be aligned unambiguously. The analysis of the genetic variation revealed that the sequences comprised 52 variable sites, 21 parsimony informative, defining 20 different haplotypes. Twelve of the 20 haplotypes were represented by only one host genotype. The remaining eight haplotypes either grouped host genotypes with a common geographical origin or showed a wide geographic distribution. The observed haplotype distribution aligned with the phylogeographic study of *D. magna* in Europe by De Gelas & De Meester (2005), which found a genetic subdivision among most populations with a few haplotypes present over a wide geographic range. The results of a neighbour-joining tree (Fig. S1, Supporting information) showed two major clusters with the far-eastern Siberian (Yakutia) group forming an isolated branch. One cluster included most Scandinavian *D. magna* clones except the ones from Åland Islands (Finland), while the other cluster consisted of host genotypes from almost the entire geographic region of the experiments. The genetic distances yielded by this analysis were used in the subsequent analysis.

INFECTION EXPERIMENTS

All control populations remained uninfected throughout the experiments. Across all three experiments, 96% of the populations exposed to parasites and 98% of the control populations survived for 25 weeks. Parasite exposure did not significantly affect population extinction (Welch's two sample *t*-test at 5 weeks: $t_{3\cdot19} = 0\cdot37$, $P = 0\cdot73$; at 25 weeks: $t_{2\cdot27} = 1\cdot11$, $P = 0\cdot37$). In experiment 1, no difference in parasite persistence (proportion infected) between low- and high-spore dose treatments was observed (GLM, $\chi^2 = 5\cdot28$, df = 1, $P < 0\cdot19$), prompting

us to conduct experiment 2 and experiment 3 with only the high dose.

All host clones from the native range of the parasite allowed persistence, whereas most clones outside this range did not (Fig. 1). Over all experiments, the geographic distance between tested host clones and parasite isolate origin correlated with the genetic distance between tested host clone and the host clone from which the parasite was isolated (Spearman rank correlation, $r = 0{\cdot}57$, $P < 0{\cdot}0001$). The geographic distance between the original site of the parasite isolate and the site of the host genotype, as well as the habitat category, showed significant effects on parasite persistence after 5 and 25 weeks (Table 1A, Fig. 2). Genetic distance between the parasite's original host genotype (source population) and the tested host genotype showed a statistically significant quadratic relationship with infection of host populations after 5 and 25 weeks (Table 1B, Fig. 2). A comparison of infections at week 5 with those at week 25 showed that the number of host clones allowing persistence became smaller with time. At least one replicate population from 31 *Daphnia* genotypes (out of 43 genotypes) was still infected by *H. tvaerminnensis* after 5 weeks, while after 25 weeks, only in replicates of 20 *Daphnia* genotypes infected populations were found. Furthermore, host clone infections showing intermediate proportions of infected replicates at week 5 almost completely disappeared by week 25, when either all or none of the replicate populations were infected (Figs 2 and 3). This was also visible in a comparison of parasite prevalences within populations at week 5. Parasite prevalence at week 5 (experiment 2 only) was significantly higher in populations of host genotypes in which *H. tvaerminnensis* persisted 25 weeks (mean = 56%, SD = 24%) than in populations that were only infected at week 5 (mean = 8%, SD = 5%) (GLM, $\chi^2 = 105{\cdot}65$, df = 1, $P < 0{\cdot}0001$). After 25 weeks, the parasite had either reached almost 100% of prevalence or disappeared.

Hamiltosporidium tvaerminnensis persisted in 67% of the tested *D. dolichocephala* populations for 25 weeks. However, none of the *D. similis*, *D. lumholtzi, D. galeata* and *D. pulex* populations were infected after 5 or 25 weeks.

Finally, we tested whether habit variables correlate with parasite persistence. Host clones from water sources with a high likelihood of going dry during the summer – whether these were rock pools in the far North, or Mediterranean ponds – strongly supported long-term parasite persistence (Fig. 3).

Discussion

Common garden experiments like the one described here allow us to assess parasite host ranges independent of environmental effects, clarifying the role of genetic factors. *Daphnia magna* genotypes exposed to a microsporidian

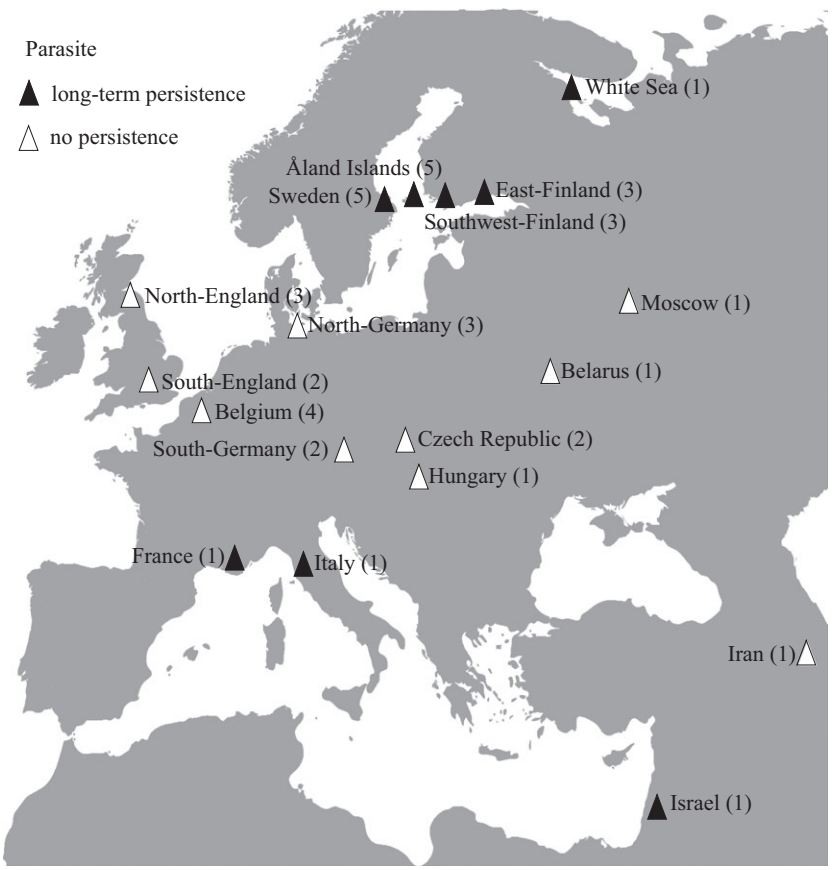

Fig. 1. Map of Europe and near Asia indicating the sites of origin of the *Daphnia magna* genotypes used. Filled triangles show sites whose host clones allowed long-term parasite persistence, while open triangles indicate lack of parasite persistence. Numbers in parenthesis indicate number of host clones used from that local region. Only one genotype was used from each population (pond, lake or pool). Only data for persistence at week 25 are shown. Sites in central Siberia (Yakutia) and Kenya (Africa) are not shown. The parasite did not persist in these host clones. The known native range of the parasite includes the rock pool populations along the Finnish and eastern Swedish coast.

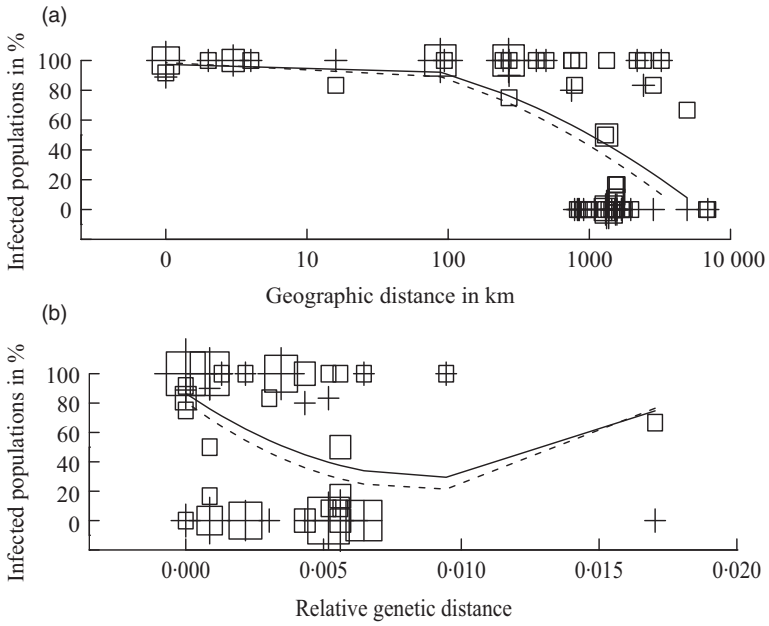

Fig. 2. Percentage of infected *Daphnia magna* replicates for all host genotype/parasite isolate combinations after 5 weeks (square) and 25 weeks (cross) plotted against (a) the geographic distance between the origin of the parasite isolate and origin of the host genotype and (b) the genetic distance between the source (native) host and the new host. Regression lines are shown for combinations after 5 weeks (dashed line) and 25 weeks (solid line). The size of the symbols indicates the number of repeated occurrences of value combinations.

parasite *H. tvaerminnensis* from different locations differed strongly in their ability to allow parasite persistence over periods long enough to include several host generations and, thus, parasite transmission cycles. Differences among host genotypes followed strong patterns, with all clonal host populations from the parasite's native geographic range allowing long-term persistence, while most clones outside this range did not – a pattern that recapitulates the well-known statistical association of declining parasite performance with increasing genetic and geographic distance from the site of parasite and host origin (reviewed in Antonovics *et al.* 2013). As opposed to the strong genetic differences observed for the host, the three parasite isolates used in the three experiments produced the same persistence results when tested in the same host clones, suggesting that they do not differ in this respect. This result is consistent with the near absence of genetic differences on DNA sequence level for this clonally reproducing parasite (Haag, Traunecker & Ebert 2013; Haag *et al.* 2013).

Surprising was our finding that all host clones from populations with a high likelihood to dry up in the summer ('summer dry') allowed long-term parasite persistence, whereas hosts from other habitats did not. Summer dryness was a nearly perfect predictor of persistence, although its geographic distribution is discontinuous, encompassing rockpool populations of the Baltic and White seas in northern Europe, as well as Mediterranean populations (France, Italy and Israel) in the South (Fig. 1). Furthermore, this result is consistent with an independent experiment with two host clones from yet another summer-dry population in Israel (A. P. Kaufmann, unpublished). The strong explanatory power of habitat type is surprising, because the common garden experimental conditions did not include ecological differences. Thus, host genotypes from summer-dry habitats carry genes that allow parasite long-term persistence. Neither our genetic analysis based on a single mitochondrial gene, nor an earlier phylogeographic study of *D. magna* (De Gelas & De Meester 2005), hints at a phylogeographic structure that could explain this result. Typically, genetic and geographic distance correlate positively in *Daphnia* (e.g. Thielsch *et al.* 2009; Jenkins *et al.* 2010). An explanation for our finding is that different host populations converged by local adaptation to their specific habitats and that genotypes locally adapted to summer-dry sites are also susceptible to *H. tvaerminnensis*. It is difficult to imagine how such a form of genetic correlation could come about. It may be that the physiological adaptations necessary to produce resting eggs that are resistant to summer dryness also influence host physiology in a way that benefits the parasite.

Our long-term persistence data give a better match to the natural distribution of *H. tvaerminnensis* than the short-term data, but the match is not perfect. Clones from the Mediterranean coasts allowed long-term persistence, although the parasite was not recorded from these areas (only the congeneric *H. magnivora*, which occurs all over Europe) (Haag, Traunecker & Ebert 2013; Haag *et al.* 2013). Furthermore, one of the five other *Daphnia* species, *D. dolichocephala*, allowed long-term persistence in four of six replicates. Such unexpected compatibilities are not unknown in novel host–parasite associations, and the development of models to predict their occurrence is a central issue in emerging disease biology (Dennehy *et al.* 2006; Pedersen & Davies 2009). Evolutionary models attribute the finding of compatible novel combinations to chance events, which are more likely to occur when the novel host is similar to the parasite's source host (Perlman & Jaenike 2003; Antonovics *et al.* 2013). Our finding of

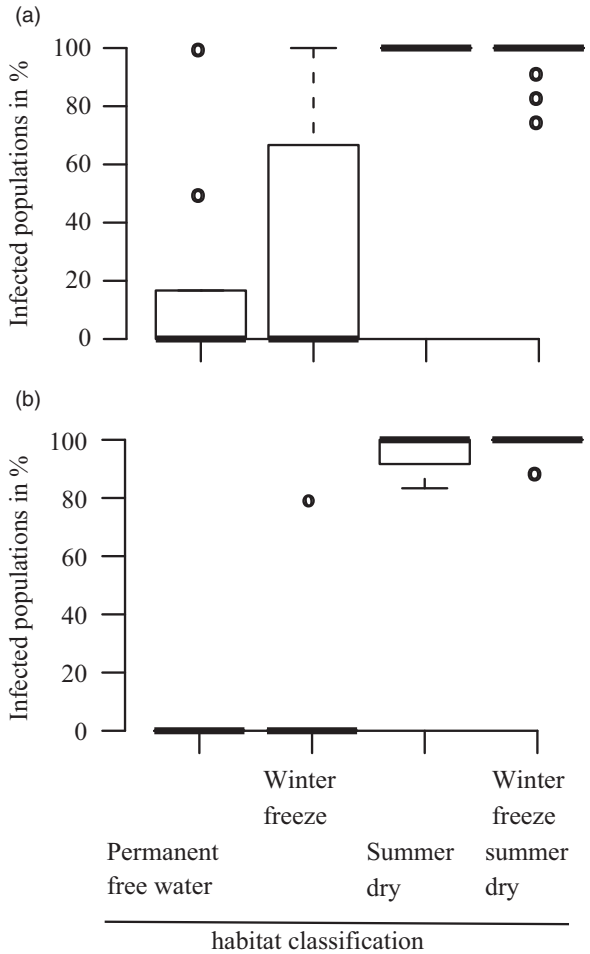

Fig. 3. Percentage of infected *Daphnia magna* populations after 5 (a) or 25 weeks (b) plotted for each host population habitat type. Box plots show medians (fat line), quartiles and 95% confidence intervals. Outliers are shown as circles.

an ecological variable explaining such exceptions for *D. magna* indicates that compatibility may also be influenced by local adaptation to factors apparently unrelated to parasitism. Interestingly, the *D. dolichocephala* clone used in this study was isolated from a summer-dry lake that holds water for only about 3 months each year (J. Mergeay, personal communication).

LONG-TERM PERSISTENCE AS A PREDICTOR FOR HOST RANGES

Parasites have to pass through a sequence of steps to complete their life cycle, each one important for parasite success (Lenski 1984; Schmid-Hempel & Ebert 2003). Interrupting a single step in this chain may block parasite transmission, and it has been hypothesized that failure to complete the transmission cycle explains the failure of some apparently infectious parasites to persist in new host populations (Woolhouse & Gowtage-Sequeria 2005). Therefore, when assessing a parasite's potential to grow and spread in a host population, one ideally includes all steps of its infection process – from the natural uptake of transmission stages to the release of transmission stages. Even this, however, may not be enough to predict parasite persistence, for even if the parasite can complete its entire life cycle, the number of transmission stages released may not be sufficient for long-term persistence. Therefore, one ideally tests parasites in experimental host populations and assesses long-term persistence. Using this approach in monoclonal host populations, we observed that *H. tvaerminnensis* can infect a wider range of host genotypes during short-term assessment (5 weeks) than it can persist in during the long term (25 weeks). Five weeks is shorter than the expected life span of infected hosts. Thus, infected animals at week 5 might have been exposed to the parasite spores in week 1. Persistence over 25 weeks is, however, only possible when host-to-host transmission occurs in the populations. Our long-term assessment replicated an estimated host range very similar to the known host range in nature, while the short-term (5-week) experiment included several host genotypes that were apparently false positive. Not including the complete transmission cycle may explain why host ranges in other experimental infection studies were typically larger than those found in nature (Futuyma, Keese & Funk 1995; Moore & Gotelli 1996; Reed & Hafner 1997; Morehead & Feener 2000; Mutikainen *et al.* 2000; Perlman & Jaenike 2003).

By using long-term persistence as a dependent variable, we were also able to reduce unexplained variation, thus increasing the explanatory power of our statistical models. First, some hosts dropped out, which supported the parasite in the short term, but not the long term, and secondly, consistency across replicate populations of the same host and parasite increased, resulting in nearly perfect bimodal distributions: host genotypes either allowed parasite persistence or did not (Fig. 3). Our data structure show that in the long-term, genetic differences among host clones become dominant, while earlier, erratic effects, possibly caused by setting up the initial populations, may contribute to the overall variation. The parasite's adaptive evolution during these times seems unlikely (as compared to similar experiments with bacteriophages (Dennehy *et al.* 2006), but cannot be ruled out. The nearly perfect bimodal distribution seen in the long-term persistence data is not seen in a previous study on individual host infection data (Ebert 2008) where within host–parasite replication of two isolates of *H. tvaerminnensis* from Finland (*Octosporea bayeri*) was assessed in 22 new host clones from three populations in Germany and nine clones from the parasite's native range. The distribution of spore counts across the novel host clones was quantitative, but on average lower than in the native host clones. This new study's results suggest that none of those 22 German host clones would have supported the parasite long term, as was the case for three host clones used in both studies.

Taken together, our results underline the need to use comprehensive measures of parasite performance to judge

the likelihood that a parasite will be able to spread in a new host population (Woolhouse & Gowtage-Sequeria 2005; Schmid-Hempel 2011). In our study, the ability of the parasite to infect a new host was likely not the issue that limited its spread in experimental host populations. Rather, a later step in the infection sequence might be the limiting factor, or a combination of weak performance at multiple steps. This observation reveals that a parasite's good performance at one step of the infection sequence cannot be used to predict its ability to spread in a host population. (Woolhouse & Gowtage-Sequeria 2005; Schmid-Hempel 2011). Our approach could be applied in other systems that allow the experimental study of host populations.

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Data accessibility

Data archived as Supporting Information.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Neighbor joining tree of 45 *Daphnia magna* clones (20 haplotypes) including spore donor clones of all three experiments constructed from 612-bp fragments of the Cytochrom oxidase 1 gene using K2P distances (bootstrapping values included). *D. magna* clones susceptible to *H. tvaerminnensis* are marked (♦).

Table S1. Clone origin coordinates and habitat information.

Table S2. GLM results of experiment 1 for the proportion of infected *Daphnia magna* populations after five and 25 weeks in dependence of (A) geographic distance (between parasite isolate and tested host genotype) and environmental conditions (four habitat types) and (B) the genetic distance between the host population from where the parasite isolate was collected from and host genotype in which the parasite was tested in. *Daphnia* genotypes that carried no infection in all experimental replicate populations at week 5 were excluded from the experiment at that point, reducing the sample size accordingly. All replicates from one clone from the White Sea (Russia), were lost before week 25. Since geographic and genetic distances are strongly confounded, it was not meaningful to include both variables in the same GLM.

Table S3. GLM results of experiment 2.

Table S4. GLM results of experiment 3.