Virulence evolution: thinking outside of the host

Luís M. Silva [1,2], Jacob C. Koella [1]

[1] Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

[2] Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada

Correspondence: Luis M. Silva (LMS): luis.silva@ubc.ca

ORCID: LMS: 0000-0002-8203-4006

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Abstract

The main theory of the evolution of virulence relies on a trade-off between virulence and transmission rate. However, it has been difficult to measure the required trade-off. A recent transmission decomposition framework explains this might be due to a lack of information about the parasite's survival in the environment outside the hosts, where the parasite finds itself during transmission between hosts. In this study, we used parasite lines of *Vavraia culicis* with varying levels of virulence upon infecting their host, the mosquito *Anopheles gambiae*, to explore the interaction between parasite-driven virulence within its host and its survival outside of the host. Our findings showed that parasite lines with greater virulence and growth within their hosts had a cost in their intrinsic ability to withstand the environment, irrespective of temperature. These results underscore the importance of considering the full context of transmission and other parasite fitness traits in studying and predicting the evolution and spread of infectious diseases.

Introduction

The evolution of virulence, defined as the decrease in the host's fitness caused by a parasite, is a major topic of debate in evolutionary biology. Understanding virulence evolution has significant implications for several fields, namely epidemiology and medicine. The dominant theory, or virulence-transmission trade-off hypothesis (Anderson & May 1982), was established over 50 years ago and has shaped our current disease control and management strategies, impacting both conservation efforts and human health (Alizon *et al.* 2009; Alizon & Michalakis 2015). This theory rests on the premise that it is inevitable to have a trade-off between virulence and the parasite transmission rate due to the growing costs of within-host parasite replication, which consequently should short host lifespan and the parasite transmission cycle (Anderson & May 1982, 1991) . Therefore, virulence is often predicted to be favoured at intermediate levels. This simple theory is both intuitive and practical and, thus, garnered support from the research community (Alizon *et al.* 2009; Bérénos *et al.* 2009; Fenner 1983; Leggett *et al.* 2017). However, in literature, we still observe limited evidence for its trade-offs and predictions from empirical and wild studies, leading to scepticism (Acevedo *et al.* 2019; Alizon & van Baalen 2005; Alizon & Michalakis 2015; Godinho *et al.* 2023; Sheen *et al.* 2024; Turner *et al.* 2021; Vallée *et al.* 2023). This uncertainty arises for several reasons.

The first reason is a direct result of the predictions raised by the theory, i.e., that highly virulent parasites will shorten their host life cycle and collaterally their own transmission cycle, reducing the number of infective cells they can produce and their chances of transmission to a new host. Evidently, such genotypes will be counter-selected and hence, in wild conditions, it will be improbable to observe such parasites. As a result, wild data mostly solely shows a positive correlation between virulence and transmission rate. The second point is how difficult it is for a simple theory to reflect the diversity in parasitic life cycles, transmission strategies and infection progression (Alizon & Michalakis 2015; Boldin & Kisdi 2012; Visher *et al.* 2021). Therefore, comparing parasites with horizontal vs. vertical transmission, direct vs. environmental, or simple *vs.* complex life cycles becomes challenging and requires that different parasites have their own set of complementary theories and predictions (Alizon & Michalakis 2015; Ben-Ami 2017). However, even by implementing these complementary hypotheses, it is not ever clear or possible to relate different parasite traits among or between each other and get the full parasite evolution picture (Boldin & Kisdi 2012; Rafaluk-Mohr 2019).

Third, and perhaps the most important, is the growing ambiguity around the correct transmission metrics, the key measure of parasitic fitness. As we know, transmission includes all steps within and between hosts (Caraco & Wang 2008; Silva *et al.* 2024). However, for simplicity or due to empirical constraints, many studies focus on specific steps. In particular, they emphasise within-host transmission evolution (often using parasite load as a proxy for transmission) while overlooking the transmission

stages between hosts, whether biotic or abiotic (Caraco & Wang 2008; Silva *et al.* 2024). Except for a few parasites (e.g., sexually transmitted ones), most parasites have a stage between hosts where they are exposed to conditions different from those within the host, potentially limiting their transmission capacity (Brandon Ogbunugafor *et al.* 2013; Caraco & Wang 2008; Rafaluk-Mohr 2019; Wasik *et al.* 2023). Although careful within-host study is vital for understanding parasite evolution, this approach restricts us from relating different steps of the parasite transmission cycle, and we end up missing the big picture. Factors, such as the host's dispersal (Arneberg *et al.* 1998; Fenton *et al.* 2002; Godinho *et al.* 2023) and the parasite's ability to withstand environmental factors (e.g., temperature or pH), (Bhardwaj & Agrawal 2020; Chatterjee *et al.* 2021b, a) can affect the parasite's ability to infect a new host (Caraco & Wang 2008) and, therefore, our predictions regarding the spread and evolution of disease.

A more inclusive framework of the factors that are likely to affect transmission ability by a parasite has been recently developed (Silva *et al.* 2024). This framework includes the whole life cycle of the parasite and, in particular, in the stage between hosts, distinguishes between the parasite's intrinsic quality to survive outside of the host (called Q_p) and the sensitivity of this quality to environmental factors (called Q_e). Hence, by decomposing transmission and quantifying and relating the factors affecting each stage, we believe we and the field can generate more comprehensive and accurate predictions of parasite evolution and gain a deeper understanding of virulence evolution as a whole (Silva *et al.* 2024).

Considering all these points, this study aimed to test this framework and examine whether it could help us understand the evolution of virulence and its relationship to transmission within and between hosts. We used the microsporidian parasite *Vavraia culicis*, which infects several species of mosquitoes (Zeferino & Koella 2024). This parasite is transmitted through spores released into water by infected individuals, which are then ingested by susceptible larvae to restart the parasite's life cycle (Becnel & Weiss 2014). A combination of the number of released spores and the longevity of the spores in the water thus determines transmission. In particular, we used parasite lines that had been selected for early or late transmission (Silva & Koella 2024) and that, therefore, had different levels of virulence.

Thus, in our study, we: i) characterised the ability of the lines to withstand the environment between hosts by measuring how their ability to infect a mosquito was affected by the time spent outside the host and by environmental temperature; ii) decomposed transmission according to the novel framework (Silva *et al.* 2024) and identified the components playing a role in the parasite's longevity in the outside-host environment; and iii) related these transmission components with their virulence (2). By considering both the time spent within and outside a host, we aim to gain a comprehensive understanding of parasite evolution and uncover inherent trade-offs that may limit its evolvability, namely concerning virulence.

Materials and Methods

Experimental model

We used the Kisumu strain of the mosquito host *Anopheles gambiae (s.s.)* and lines of the microsporidian parasite *Vavraia culicis floridensis* selected for seven generations: i) for early transmission and shorter time within the host or for late transmission and longer time within the host; ii) or lines taken from our unselected stock as a baseline reference (Silva & Koella 2024). Further details on the selection experiment and its maintenance are described in Silva and Koella (2024). Experiments and rearing were conducted at standard laboratory conditions (26 ± 1ºC, 70 ± 5% relative humidity and 12h light/dark).

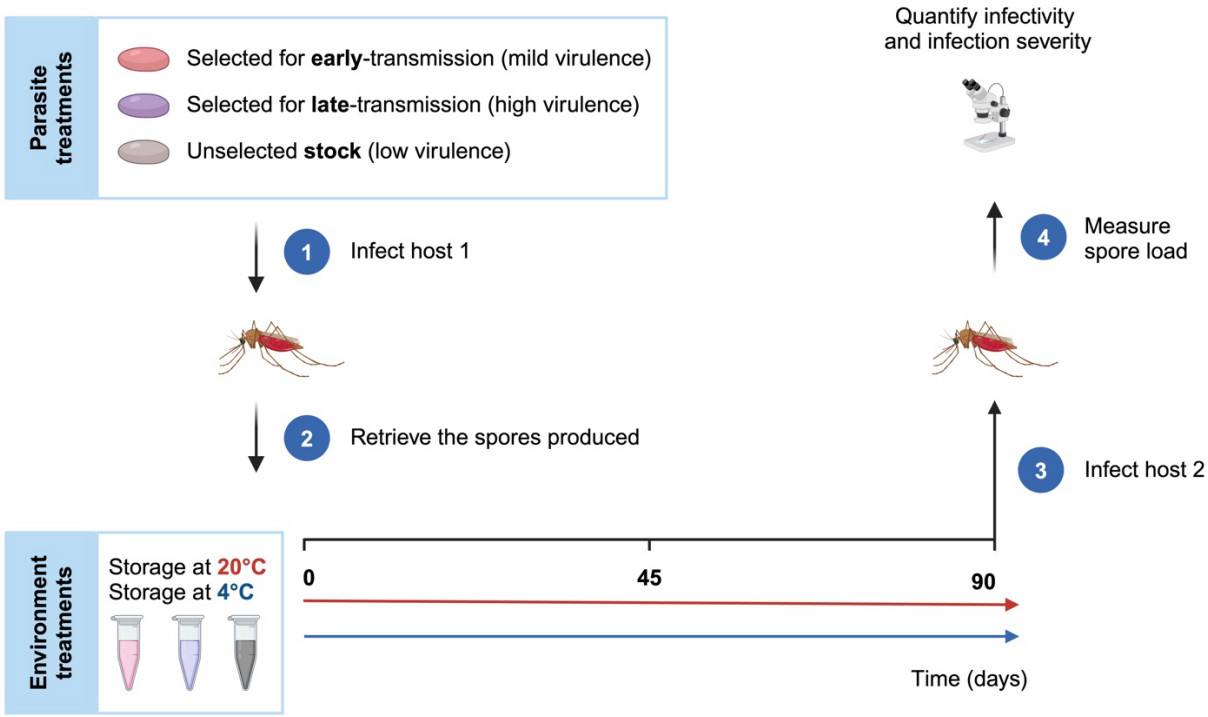

Figure 1. Experimental design. Summary illustration of the experimental design and respective workflow. In brief, different microsporidian parasite *V. culicis* lines were used to infect *A. gambiae* mosquitoes. The spores were stored at one of two temperatures (i.e., 4 ºC or 20 ºC) for 0-, 45- and 90-days post-spore-production and then used to infect naive *A. gambiae* mosquitoes. Spore load was then measured at day 10 of adulthood to assess the infectivity and infection severity of the different parasite lines. Figure generated with Biorender.com.

Preparation of parasite aliquots

Freshly hatched *A. gambiae* larvae were reared in groups of 50 per Petri dish (120 mm diameter x 17 mm) containing 80 ml of distilled water. They were fed daily with Tetramin Baby fish food according

to their age: 0.04, 0.06, 0.08, 0.16, 0.32 and 0.6 mg/larva for 0, 1, 2, 3, 4 and 5 days or older, respectively. Two days after hatching, larvae were exposed to 10,000 spores of *V. culicis* per larva, from spores obtained parasites selected for early-transmission, late-transmission, or not selected (i.e., stock). Each treatment consisted of five replicate lines maintained in similar conditions. Upon pupation, individuals were transferred to cages (21 x 21 x 21cm) and left to emerge as adults. The adults were provided constant access to a 6% sucrose-water solution. Dead mosquitoes were collected shortly after their death until day 20 (when the experiment ended), and the remaining mosquitoes were collected. The collected mosquitoes were put into 2ml microcentrifuge tubes with 1ml of distilled water, with at most 25 mosquitoes per tube, and kept at 4ºC and in the dark. At the end of the collection, a stainless-steel bead (Ø 5 mm) was added to each of the tubes and the mosquitoes were homogenized with a Qiagen TissueLyser LT at a frequency of 30 Hz for two minutes. The resulting homogenate containing the parasite spores was transferred to a 15 ml falcon tube, and the spore concentration was estimated with a haemocytometer under a phase-contrast microscope (400x magnification). From each falcon tube, we prepared aliquots with a spore concentration of 500,000 spores/ml for the infections in the following experiment. The resulting aliquots contained spores in 1:1 distilled water and an antibiotic-antimycotic cocktail solution (Sigma-Aldrich A5955, containing 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin per ml, filtered), which limits the growth of contaminating microbes without affecting microsporidia (Van Gool *et al.* 1994; Jaronski 1984; Visvesvara 2002; Visvesvara *et al.* 1999). A preliminary experiment was run to ensure that the antibiotic-antimycotic solution did not interfere with the production or development of the parasite's spores. Aliquots were kept in the dark at 4 ± 0.5 ºC or 20 ± 0.5 ºC dark until they were used.

Infectivity and infection severity experiment

To assay how time in the external environment and temperature of the environment affect infectivity, we infected mosquitoes (reared as described above) with one of the aliquots prepared 0, 45, or 90 days earlier (Fig. 1). We used each aliquot only once to avoid contamination. After pupation, mosquitoes were moved to individual 50 ml falcon tubes containing approximately 10 ml of distilled water (Dao *et al.* 2010), and at adult emergence, they were transferred to individual cups (5 cm Ø x 10 cm) with access to a 6% sugar solution and a humid filter paper. Ten days after emergence, the mosquitoes were frozen at -20ºC for later spore counts. This time point was chosen as all mosquitoes infected with any of the parasite lines have started to produce spores (Silva & Koella 2024).

Statistical analysis

The analyses were performed with R version 4.3.1 in RStudio version 3034.06.2+561. The packages "DHARMa" (Hartig & Hartig 2017), "car" (Fox *et al.* 2012), "lme4" (Bates *et al.* 2009) and "emmeans"

(Lenth *et al.* 2019) were used to analyse the data. We used the packages "ggplot2" (Wickham 2016) and "scales" (Wickham *et al.* 2022) to plot the data and Biorender.com to assemble the figure panels.

Spore infectivity was measured as the proportion of infected individuals. Hence, we assessed differences in infectivity using a generalised linear mixed model with a binomial error, parasite selection, time in the external environment, temperature, and all interactions as explanatory variables. The selection line was included as a random factor. Infection severity was defined as the spore load of individuals containing spores. It was analysed with a linear mixed model with a Gaussian error, the same explanatory variables, and a random factor as infectivity.

Following the framework developed by Silva *et al.* (2024), we then quantified differences in spore quality (Q_p) and environmental effect (Q_e) for spore infectivity and infection severity (Silva *et al.* 2024) (Fig. 3ab). Q_p for infectivity was calculated as the difference within each selection line of infectivity (proportion of individuals with spores) at 0 days and 90 days (Fig. 3a). Q_p for infection severity was calculated as the difference of spore load obtained after 0 days and after 90 days (Fig. 3b). As our standard environment we considered 4ºC, the temperature the spores have been stored to for several years before this experiment. In both cases, we used a linear mixed model with parasite treatment as the explanatory variable and the replicate as a random factor. Q_e for infectivity was calculated as the difference of infectivity between 4ºC and 20ºC for the time-point of 90 days, where we observe the most striking difference due to temperature (Fig. 3b). Q_e for infection severity was the difference of the average spore load obtained after 90 days between parasite kept at 4 ºC and 20ºC days (Fig. 3b). We analysed both using a linear mixed model with parasite treatment as the explanatory variable and the replicate as a random factor.

We used the maximum hazards calculated by Silva and Koella (2024) as proxies for infection virulence in the first host. We related virulence to infectivity by assessing whether the virulence of each selection line explained the differences in Q_p or Q_e for infectivity or infection severity. This was done using linear models, where the dependent and the independent variables were transformed by adding one and taking the logarithm. The curves in Fig. 4a-d were calculated using the respective lm function.

Results

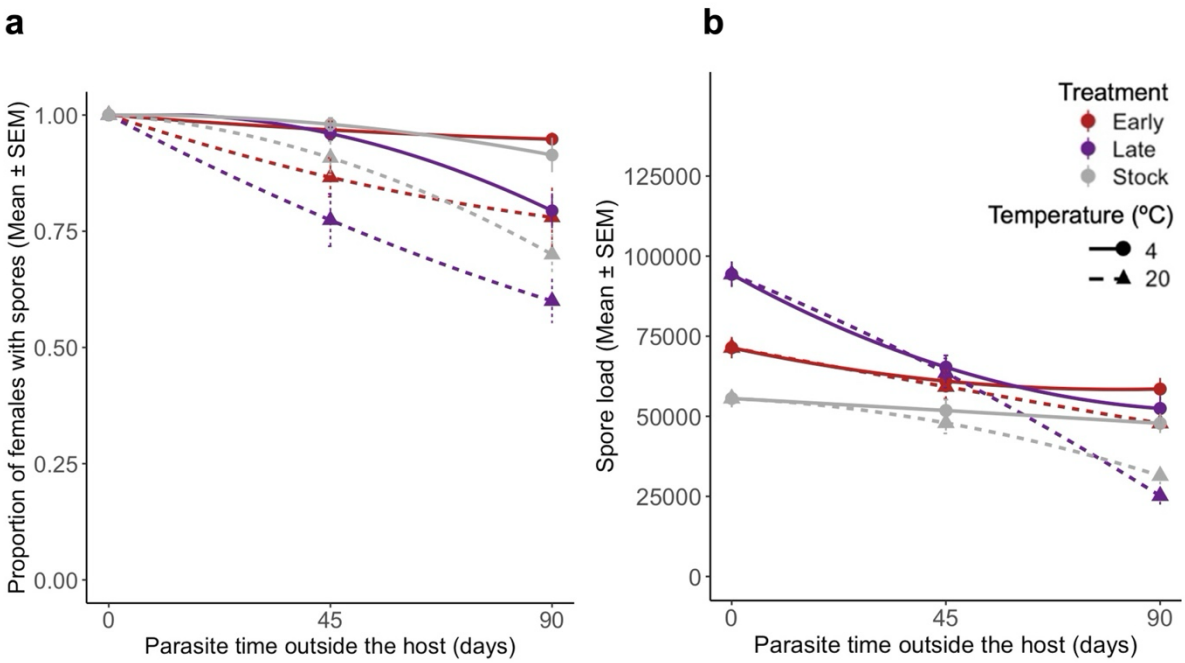

Figure 2. Parasite performance in a new host. (a) Infectivity of the spores by regime, time and storage temperature. The latter was defined as the proportion of successful infections by day 10 of adulthood. Each mean consists of 150 females per treatment, time and temperature, split through five replicates. **(b)** Infection severity of the females carrying spores for the same time-point, as the number of spores per female. The sample size is the following for 4ºC and 20ºC, respectively. Day 0: All treatments consisted of 150 females. Day 45: Early with 145 and 130, Late with 144 and 116, Stock with 147 and 136. Day 90: Early 142 and 117, Late 119 and 90, Stock with 137 and 105.

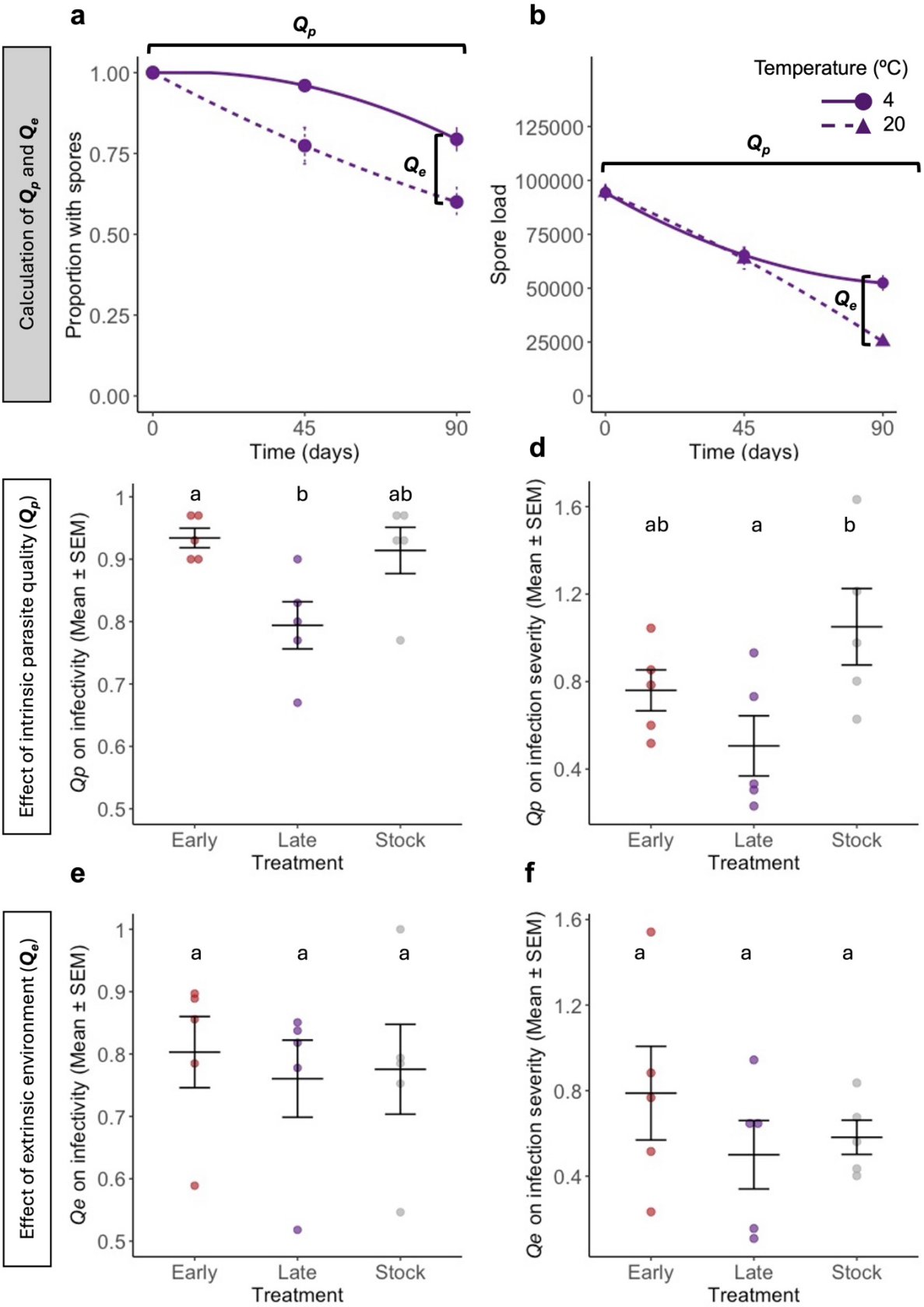
Calculation of Q_p and Q_e
a
Q_p
Q_e
Proportion with spores
1.00
0.75
0.50
0.25
0.00
0
45
90
Time (days)
b
Temperature (ºC)
4
20
Q_p
Q_e
Spore load
125000
100000
75000
50000
25000
0
0
45
90
Time (days)
Effect of intrinsic parasite quality (Q_p)
Qp on infectivity (Mean ± SEM)
a
b
ab
Early
Late
Stock
Treatment
d
Qp on infection severity (Mean ± SEM)
ab
a
b
Early
Late
Stock
Treatment
Effect of extrinsic environment (Q_e)
e
Qe on infectivity (Mean ± SEM)
a
a
a
Early
Late
Stock
Treatment
f
Qe on infection severity (Mean ± SEM)
a
a
a
Early
Late
Stock
Treatment

Figure 3. Calculation of the *Qp* and *Qe* for infectivity and infection severity. Parasite quality (Q_p) and environment (Q_e) effects on infectivity **(a)** and infection severity **(b)** were estimated, as demonstrated in the figures. In these figures, we demonstrate this rationale using the late-selected spores' infectivity and infection severity curves per environmental temperature and by time post-spore production presented in Figure 1. Q_p was measured as the mean difference in the proportion of females with spores/spore load between infection with 90-days and 0-days old spores at 4º C. Q_e, as the effect of environment temperature in this model, was calculated by subtracting the mean proportion of females with spores/spore load after exposure to spores kept at 20ºC for 90 days from the mean proportion of females with spores/spore load at 4ºC for 90 days. The resulting means of each replicate from each treatment were then used in Figures 2c-e. The effects of parasite selection on the parasite spore quality (Q_p) and environmental temperature (Q_e) for **(ce)** infectivity and **(df)** infection severity (i.e., spore load). Each data point represents the mean value of one of the five replicated selection lines.

The direction of selection affected the parasite's ability to establish an infection in a new host, with stock and early-selected parasites being more infective than late-selected parasites ($\chi2 = 33.00$, $df = 2$, $p < 0.001$; Fig. 2a). The longer the spores were kept in the external environment, the less infective they were ($\chi2 = 77.56$, $df = 1$, $p < 0.001$). If they were kept in the external environment at 20ºC, they were less infective than if they were kept at 4ºC ($\chi2 = 166.85$, $df = 1$, $p < 0.001$; Fig. 2a). None of the interactions between selection treatment, temperature and duration in the external environment was significant. A similar pattern was observed for spore load (infection severity) of established infections (Fig. 2b) (selection: $\chi2 = 135.31$, $df = 2$, $p < 0.001$; duration in the external environment: $\chi2 = 14.23$, $df = 2$, $p < 0.001$; temperature: $\chi2 = 4.46$; $df = 1$; $p = 0.035$). The effect of the duration in the external environment (interaction selection * duration: $\chi2 = 45.51$, $df = 2$, $p < 0.001$) and of temperature (interaction selection * temperature: $\chi2 = 7.35$; $df = 1$; $p = 0.025$) was more pronounced for parasites selected for late transmission than for early-selected or unselected parasites.

The component of intrinsic quality, Q_p, was significantly linked to the average virulence of a selection line (Figs. 3ab), with more virulent lines being less able to infect a new host ($\chi2 = 13.00$; $df = 2$; $p < 0.001$) and producing fewer spores in the new host ($\chi2 = 4.58$; $df = 2$; $p = 0.032$) after a long time in the external environment. However, the environmental sensitivity component, Q_e, was not linked to virulence (Fig. 4cd).

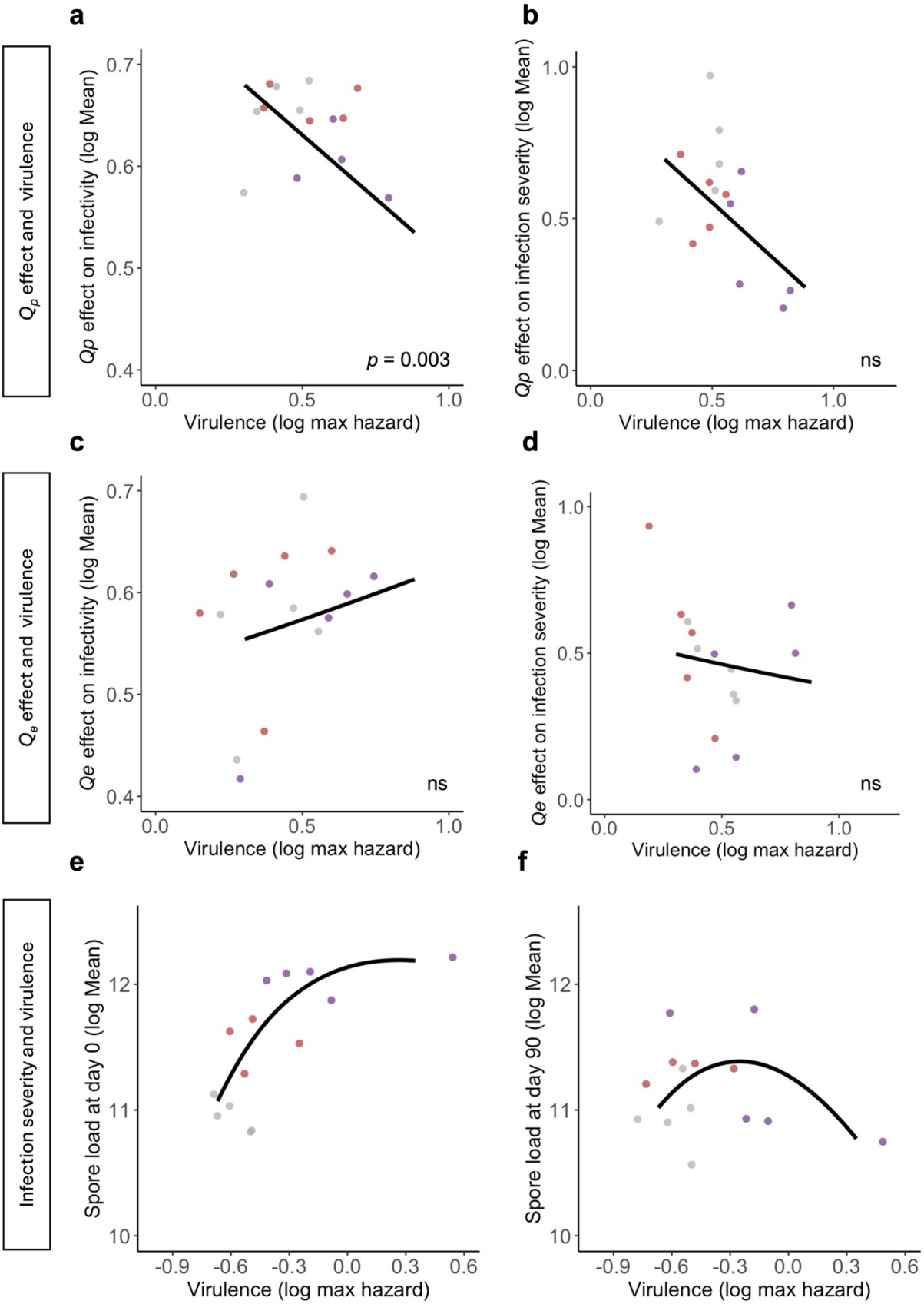
a
b
c
d
e
f
Q_p effect and virulence
Q_e effect and virulence
Infection severity and virulence
Qp effect on infectivity (log Mean)
Qp effect on infection severity (log Mean)
Qe effect on infectivity (log Mean)
Qe effect on infection severity (log Mean)
Spore load at day 0 (log Mean)
Spore load at day 90 (log Mean)
Virulence (log max hazard)
p = 0.003
ns
ns
ns
0.7
0.6
0.5
0.4
1.0
0.5
0.0
12
11
10
0.0
0.5
1.0
-0.9
-0.6
-0.3
0.0
0.3
0.6

Figure 4. Virulence-transmission decomposition. Parasite's virulence in the first host in relationship to Q_p *and* Q_e effect on infectivity **(ac)** and infection severity **(bd)** in a new host, respectively. Each point represents the average values of a replicated selection line. The curves show the result of the linear model. Parasite burden in a new host is caused after a time between hosts of 0 **(e)** and 90 days postproduction **(f)**. In these last two plots, a smooth line with the "loess" method was generated for ease of visualisation.

Discussion

Most parasites spend some of their life cycle outside the host when transmitting from one host to the next. While this phase of parasitic life cycles is often ignored in evolutionary ideas, it has been considerably studied and described concerning viruses (Kormuth *et al.* 2019; Kotwal & Cannon 2014) and parasites with complex life cycles (Bradley *et al.* 2018; Davies *et al.* 2001). Here, we suggest that integrating and relating within- to outside-host parasite evolution may lead to important insights, mainly if the efficacy of transmission during the period spent outside the host trades off with growth and virulence within the host. A standard prediction, for example, is that selecting for later transmission would result in slower parasite growth within the host and lower virulence. However, an experiment with the artificial selection lines of the parasite *V. culicis* selected for early or late transmission showed the opposite pattern (Silva & Koella 2024). As discussed by the authors, this unexpected result could arise from the fact that the experimental design eliminated any costs of virulence during the transmission phase in the external environment between hosts. This experimental design favoured the selection of parasites that invest in exploitation and survival within the host rather than outside of it (Silva & Koella 2024). This study's findings corroborate this idea and highlight the importance of considering transmission as a whole in parasite evolution (Boldin & Kisdi 2012; Silva *et al.* 2024; Visher *et al.* 2021).

First, the longer the spores were kept outside of a host, the less able they were to infect a new host (Fig. 2a). This result was particularly notable in the lines with the highest growth within the host (that is, the lines selected for late transmission), indicating a trade-off between growth and virulence within the host and survival outside of the host. Other studies have suggested a trade-off between virulence and survival either outside of the host or between hosts (Davies *et al.* 2001; Dearsly *et al.* 1990; Leggett *et al.* 2014), and indeed, adaptation to one environment (e.g., within the host) is generally expected to incur costs in other environments (e.g. outside of hosts) (Caraco & Wang 2008). Similar results have been observed in viral studies when viruses are subjected to different environments throughout their transmission cycle (Bhardwaj & Agrawal 2020; Brandon Ogbunugafor *et al.* 2013; Wasik *et al.* 2023). Infection severity in the next host (that is, spore load) showed similar results from infectivity (Fig. 2b). This result suggests that the mortality of spores leads to less effective infectivity, which in turn results in a smaller dose of

infection and thus (as observed in other parasites) slower within-host growth later on (Duneau *et al.* 2017; Hidalgo *et al.* 2022; Torres *et al.* 2016). Additionally, since infection severity decreases more rapidly than infectivity between 45- and 90-day-old spores in the external environment, time outside the host affects not only the survival of the infective spores but also their infection potential. This suggests that an additional process is at play. One possibility is that the resources within a spore may be consumed or degraded over time, which could delay its growth upon entering the host. Such a process, called growth arrest, has not been described for microsporidia but is common in bacteria (Bergkessel *et al.* 2016; Hobbie & Hobbie 2013). It helps the bacteria meet their energetic and metabolic requirements before restarting replication when cells become more susceptible to environmental stressors (Moreno-Gámez *et al.* 2020). Another hypothesis is that parasites with free-living stages invest in processes and metabolic changes that allow them to subsist harmful abiotic factors (e.g., ultraviolet radiation and chemical exposure) for a considerable time. Such changes in metabolism require time and the suitable precursors and signalling to be reversed, which might hinder their instantaneous growth within a new host (Caraco & Wang 2008; Leblanc & Lefebvre 1984).

Second, the decrease of infection success in a new host with time spent outside of a host was stronger when the spores were kept at 20ºC than when they were kept at 4ºC (Fig. 1). Reduced spore survival at higher temperatures has been described for other aquatic microsporidia species, which thrive at lower temperatures (Becnel & Weiss 2014). In addition to their natural ecology, the parasite used in our experiment has been stored at cold temperatures, for in our lab, it is stored in the fridge (i.e., at 4 ºC). Again, we cannot discard the fact that adaptation to a colder environment might have happened. Nevertheless, literature on aquatic microsporidia suggests they naturally survive better at lower temperatures (Becnel & Weiss 2014).

Third, by decomposing the parasite's transmission success across different selection lines, an unselected parasite population, and temperatures, we were able to disentangle the extrinsic effect of the environment (Q_e) and the parasite's intrinsic quality (Q_p) on infectivity and infection severity (Fig. 3) (Silva *et al.* 2024). This allowed us to describe how each of these components relates to parasite's virulence within the host. While Q_e effect on infectivity or infection severity seems unrelated to any treatment, Q_p was. Thus, late-selected spores were less resistant to time outside the host than early-selected and control ones. Still, this resilience was intrinsic to the parasite and not affected by temperature (Fig. 3). Furthermore, Q_p, but not Q_e for both measures of transmission were negatively correlated with virulence (Fig. 4ab), suggesting a trade-off between virulence and parasite endurance in the external environment and corroborating that some microbes have a trade-off between reproduction and survival of their offspring generations. Such a hypothesis has been highly debated in the literature (Bonhoeffer *et al.* 1996; Caraco & Wang 2008; Rafaluk-Mohr 2019; Silva *et al.* 2024), as there are examples of parasites with high virulence and low persistence in the outside environment, as

well as very high (Rafaluk-Mohr 2019). The last strategy is named the Curse of the Pharaoh hypothesis, which states that virulence and persistence in the environment might be disentangled (Bonhoeffer *et al.* 1996) in specific ecological conditions. The parasite's prevalence in an environment could be secured in this scenario, even in fluctuating host numbers. Hence, depending on the ecological circumstances of the host-parasite relationship, we can observe both patterns in nature (Rafaluk-Mohr 2019). Nevertheless, in our case, and likely most parasites, *V. culicis* is a self-sustaining parasite that can colonise multiple hosts and prevails in different host populations (Becnel & Weiss 2014; Zeferino & Koella 2024), which suggests a considerable genetic diversity that would not justify investing in the strategy described by the Curse of the Pharaoh (Bonhoeffer *et al.* 1996). In this case, evolution should favour pleiotropy constraints between virulence and persistence outside of the host, such as the negative relationship observed in this study (Fig. 4).

Overall, our findings emphasize the importance of considering both ecology and the whole parasitic transmission cycle when studying parasite and virulence evolution (Carlsson & Råberg 2024; Silva *et al.* 2024). By quantifying parasite survival to different environmental parameters, we could better explain the evolution of virulence in this system. Moreover, by using the transmission framework, we could associate this cost in parasite longevity outside of the host to the intrinsic properties of the parasite. The assumptions and conclusions of this study are of general importance. They can be applied to several taxa, as they reflect most of the parasite's transmission cycles and consequent evolutionary paths. In fact, when we glance over the relationship between infection severity in a new host and virulence, we observe one of the advantages of having the full picture over transmission. While infection severity with freshly produced spores has a linear relationship with virulence (Fig. 4e), the same trait with 90-day-old spores produces a concave shape (Fig. 4f), as expected from the virulence-transmission trade-off theory. Hence, we believe the approach taken in this study undeniably reflects the complex network between transmission and other parasite fitness traits, as well as the limitations and possible negative consequences of only looking at within-host parasite fitness. The study and dissection of this network and their inherent trade-offs have major ramifications for studying disease ecology and parasite evolution. We can only better predict disease spread and plan consequent control strategies by understanding the latter. For instance, the findings in this study alert us that certain social behaviours (e.g., crowding) or social settings directly affecting susceptible host density might reduce the parasite time outside of the host, which in turn might inadvertently select for more virulent strains within a new host.

Author contributions

LMS conceived the overall idea, JCK and LMS designed the experiments, LMS collected and analysed the data, and LMS wrote the first draft of the manuscript. Both authors contributed critically to the drafts.

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Supplementary information

Table S1. Parasite infectivity and infection severity in a new host.

Table S2. *Qp* and *Qe* on infectivity and infection severity.

Table S3. Virulence-transmission decomposition.

Table S1. Parasite infectivity and infection severity in a new host.

Tested effect			
Model 1a - Proportion of females with spores	**χ2**	***df***	***p***
Treatment	32.996	2	**< 0.001**
Temperature	77.557	1	**< 0.001**
Time	166.849	1	**< 0.001**
Treatment: Temperature	1.011	2	0.603
Treatment: Time	3.446	2	0.179
Temperature: Time	0.433	1	0.511
Treatment: Temperature: Time	1.867	2	0.393
Model 1b - Spore load	**χ2**	***df***	***p***
Treatment	135.307	2	**< 0.001**
Temperature	0.002	1	0.961
Time	14.228	1	**< 0.001**
Treatment: Temperature	0.814	2	0.666
Treatment: Time	45.506	2	**< 0.001**
Temperature: Time	4.459	1	**0.035**
Treatment: Temperature: Time	7.352	2	**0.025**

Table S2. *Qp* and *Qe* on infectivity and infection severity.

Tested effect			
Model 2a - *Qp* on infectivity	**χ2**	***df***	***p***
Treatment	11.286	2	**0.004**
Multiple comparisons for Model 2a		***t*-ratio**	***p***
Early - Late		3.106	**0.035**
Early - Stock		0.444	0.900
Late - Stock		-2.662	0.067
Model 2b - *Qp* on infection severity	**χ2**	***df***	***p***
Treatment	9.022	2	**0.011**
Multiple comparisons for Model 2b		***t*-ratio**	***p***
Early - Late		1.884	0.205
Early - Stock		-1.083	0.550
Late - Stock		-2.968	**0.043**
Model 2c - *Qe* on infectivity	**χ2**	***df***	***p***
Treatment		2	
Model 2d - *Qe* on infection severity	**χ2**	***df***	***p***
Treatment	1.809	2	0.405

Table S3. Virulence-transmission decomposition.

Tested effect			
Model 4a - *Qp* on infectivity *vs.* virulence	**F**	***df***	***p***
log(Maximum hazard +1)	12.934	1	**< 0.001**
Model 4b - *Qp* on infection severity *vs.* virulence	**F**	***df***	***p***
log(Maximum hazard +1)	4.351	1	0.057
Model 4c - *Qe* on infectivity *vs.* virulence	**F**	***df***	***p***
log(Maximum hazard +1)	0.464	1	0.508
Model 4d - *Qe* on infection severity *vs.* virulence	**F**	***df***	***p***
log(Maximum hazard +1)	0.155	1	0.701