Transgenerational plasticity alters parasite fitness in changing environments

Syuan-Jyun Sun [1,2,*], Marcin K. Dziuba [1], Kristina M. Mclntire [1], Riley N. Jaye [1], and Meghan A. Duffy [1]

[1]Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA; [2]International Degree Program in Climate Change and Sustainable Development, National Taiwan University, Taipei 10617, Taiwan

* **Author for correspondence:** Syuan-Jyun Sun, E-mail: sjs243@ntu.edu.tw

Abstract

Transgenerational plasticity can help organisms respond rapidly to changing environments. Most prior studies of transgenerational plasticity in host-parasite interactions have focused on the host, leaving us with a limited understanding of transgenerational plasticity of parasites. We tested whether exposure to elevated temperatures while spores are developing can modify the ability of those spores to infect new hosts, as well as the growth and virulence of the next generation of parasites in the new host. We exposed *Daphnia dentifera* to its naturally co-occurring fungal parasite *Metschnikowia bicuspidata,* rearing the parasite at cooler (20°C) or warmer (24°C) temperatures and then, factorially, using those spores to infect at 20°C and 24°C. Infections by parasites reared at warmer past temperatures produced more mature spores, but only when the current infections were at cooler temperatures. Moreover, the percentage of mature spores was impacted by both rearing and current temperatures, and was highest for infections with spores reared in a warmer environment that infected hosts in a cooler environment. In contrast, virulence was influenced only by current temperatures. These results demonstrate transgenerational plasticity of parasites in response to temperature changes, with fitness impacts that are dependent on both past and current environments.

Key words: transgenerational plasticity, host-parasite interactions, climate change, *Daphnia dentifera*, *Metschnikowia bicuspidata,* priming

Key Findings

- Parasite fitness was affected by both rearing and current temperatures in a *Daphnia*-parasite system.
- Warm rearing and current temperatures induced higher total spore production.
- Rearing and current temperatures interacted to determine mature spore yield per host; infections by spores reared at warmer temperatures produced more viable spores at cooler current temperatures.
- In contrast, parasite virulence was influenced by current temperature but not rearing temperature.
- These findings demonstrate that transgenerational plasticity can influence parasite fitness.

Introduction

In the face of rapid environmental changes, avoiding extinction requires organisms to respond with behavioral changes, moving to a new geographic region, rapid evolution, and/or phenotypic plasticity (Hoffmann and Sgrò, 2011; Munday *et al.*, 2013; Wong and Candolin, 2015; Radchuk *et al.*, 2019). For organisms with limited behaviors and mobility, phenotypic plasticity has the potential to be particularly important, as it allows for a rapid response without genetic changes. Thus, for many organisms, phenotypic plasticity is expected to play a key role in allowing organisms to cope with rapidly changing environments (Charmantier *et al.*, 2008; Wong and Candolin, 2015).

Moreover, phenotypic plasticity can facilitate rapid evolutionary processes, including by buying time for genetic adaptations to occur (Mousseau and Fox, 1998; Sun *et al.*, 2020). This can be particularly true when a plastic response to environmental changes can be passed from one generation to the next, something known as transgenerational plasticity or maternal effects

(Harmon and Pfennig, 2021). Transgenerational plasticity has been demonstrated in many taxa; often, previous generations 'prime' their offspring for environmental conditions that match those of maternal generations (Mousseau and Fox, 1998; Burgess and Marshall, 2014). If the parental environments are accurate predictors for the environments of offspring, transgenerational plasticity can be adaptive, increasing the fitness of offspring experiencing those environmental conditions (Galloway and Etterson, 2007).

Although transgenerational plasticity is increasingly considered as a potentially important mechanism to counteract the negative impacts of environmental changes, most studies have focused on responses of a single species to changing abiotic environments, such as temperature elevation (Shama *et al.*, 2014) or environmental pollution (Tran *et al.*, 2019; Meng *et al.*, 2021). However, changing environments also have the potential to influence the way organisms interact. Focusing on just one type of interaction, host-parasite interactions, increasing temperatures can alter parasite reproduction, infectivity and prevalence, host resistance, and ultimately epidemic dynamics (Harvell *et al.*, 2002; Altizer *et al.*, 2013; Gehman *et al.*, 2018). While this likely leads to selection on parasites in many cases, transgenerational plasticity may also play a role in allowing parasites to persist in a rapidly changing environment. There have been some studies of transgenerational plasticity in host-parasite interactions, but most of the attention has focused on hosts (Pigeault *et al.*, 2015; Gervasi *et al.*, 2015; Nystrand *et al.*, 2016; Michel *et al.*, 2016; Roth and Landis, 2017; Paraskevopoulou *et al.*, 2022) rather than the parasite or the overall outcome of host-parasite interactions (Tseng, 2006; Little *et al.*, 2007; Shocket *et al.*, 2018). This means that, at present, there is a major gap in our understanding of how host-parasite interactions and parasite fitness change as environments change, hindering our ability to understand and predict epidemic dynamics in a rapidly changing world.

In addition to its importance in light of anthropogenic climate change, understanding transgenerational plasticity of parasites is important because seasonal changes in environments

86 are common and can influence the outcomes of host-parasite interactions within a generation
87 (Altizer *et al.*, 2006; Martinez, 2018). Changing temperatures can have complex impacts on
88 hosts and parasites (Altizer *et al.*, 2013), making it difficult to predict *a priori* how parasitism
89 should change seasonally (Altizer *et al.*, 2006). One factor that increases the complexity even
90 further is that hosts might encounter parasites that were produced in previous seasons (or even
91 years) under different environmental conditions (Decaestecker *et al.*, 2004; Shocket *et al.*,
92 2018). Overall, it is clear that: changes in temperature can strongly impact host-parasite
93 interactions; in some systems, hosts encounter parasites that were produced at different
94 temperatures; and the conditions under which a parasite develops can impact its infectivity.
95 However, at present, it is not clear whether parasite transgenerational plasticity influences key
96 parasite traits such as virulence.
97 In this study, we tested whether the temperature at which parasite spores develop
98 influences performance of their offspring using a two-generational laboratory experiment in a
99 zooplankton-fungal system. *Metschnikowia bicuspidata* is a fungal parasite commonly found
100 infecting the zooplankton host *Daphnia dentifera* in freshwater lakes in Northern America
101 (Cáceres *et al.*, 2014). In this system, *D. dentifera* is exposed to *M. bicuspidata* spores during
102 filter-feeding. The ingested needle-shaped spores then penetrate through the gut barrier into the
103 body cavity (Stewart Merrill *et al.*, 2019), where the spores develop and reproduce, eventually
104 killing the host (Ebert, 2005). Upon host death, the spores of the next generation are released
105 into the environment, where they can be ingested by new hosts to complete the infection cycle
106 (Ebert, 2005). Previous work shows that *D. dentifera* and *M. bicuspidata* are likely to encounter
107 each other during autumnal epidemics: infection prevalence tends to increase when
108 temperatures cool from 25°C in the late summer, with infection prevalence peaking at 20°C,
109 and decreasing as temperatures drop during winter (Shocket *et al.*, 2018). Moreover, it is likely
110 that spores overwinter in the sediment, with some of these becoming resuspended and infecting

new hosts in future years (Ebert, 1995; Decaestecker *et al.*, 2002); this means that spores produced at the end of one season (in colder conditions) might infect a host at warmer temperatures in a subsequent year. Thus, we would expect that *D. dentifera* can be exposed to *M. bicuspidata* spores derived from warmer or cooler thermal conditions. If transgenerational plasticity exists, we predict that parasites reared at different temperatures should perform differently; if such transgenerational effects are adaptive, we predict that offspring will perform better when their temperature matches that of their parent. In contrast, if transgenerational plasticity is not important, only the current temperatures, rather than rearing temperatures, should influence parasite performance. Prior studies have shown that rearing environments (namely: host genotype and temperature) can influence the infectivity of *M. bicuspidata* spores (Searle *et al.*, 2015; Shocket *et al.*, 2018); this shows plasticity of parasites but not transgenerational plasticity, given that the spores from the rearing conditions are the same ones that infect the new hosts. Here, we tested for transgenerational plasticity impacting parasite growth and virulence.

Materials and methods

Experimental design

We conducted a fully factorial experiment by exposing *D. dentifera* hosts to *M. bicuspidata* spores: the spores were reared either at cooler (20°C) or warmer temperatures (24°C) and then used factorially for exposures at 20°C or 24°C. The 4°C elevation of temperatures that we studied is in line with the predicted climate change scenario by the end of this century (Beits *et al.*, 2011). It also is well within the range of changes in temperature in the upper mixed layer of lakes that occur during epidemics (Shocket *et al.*, 2018). In total, this resulted in four treatment combinations of rearing and current temperatures, with 40 replicates per treatment.

Laboratory stocks of *D. dentifera* and *M. bicuspidata* originated from lakes in Barry County in Michigan, US.

Because infections will occur at the ambient temperature, and because hosts will have been reared at that temperature, we reared hosts for this experiment at the two focal temperatures. More specifically, we reared *D. dentifera* at 20°C or 24°C in only two separate incubators (I-41VL, Percival Scientific) for two generations on a 16:8 photoperiod; this means that temperature treatment is confounded with incubator (as is common in this type of experiment due to logistic constraints). We then collected neonates aged 1-2 days old. Each neonate was kept individually in a 50 mL beaker filled with 50 mL filtered lake water and was fed three times a week with a phytoplankton food (*Ankistrodesmus falcatus*, 20,000 cells/mL).

To create sources of *M. bicuspidata* spores from different temperatures, we infected *D. dentifera* individually by adding spores at a density of 145 spores/ml at 20°C and 24°C. We collected the dead hosts upon natural death, stored them individually in a 1.5 mL tube filled with 100 μL deionized water, and placed tubes in a refrigerator before use (*M. bicuspidata* spores die when placed in a freezer (Duffy and Hunsberger, 2019)). All spores used for the experiment were derived from hosts that had died 1-2 months prior to use in infections; these infected hosts were the product of an earlier experiment (Sun *et al.*, 2022). Infected hosts were ground individually using electric pestles for 60 seconds before we infected new hosts with a well-mixed solution of spores (145 spores/ml) from each tube at 20°C and 24°C. We used a degree-day approach (Vale *et al.*, 2008; Manzi *et al.*, 2020) by adding spores to *Daphnia* at an age of 6 and 5 days, for 20°C and 24°C, respectively, resulting in a 120 degree-day. At exposure, we fed all animals 20,000 cells/mL *A. falcatus* and kept them at a 16:8 light:dark cycle. Thereafter, all animals were fed *A. falcatus* (20,000 cells/mL) three times a week. We terminated the experiments when all animals had died. This experiment was conducted in March 2022.

160

161 *Data collection*

162 To determine virulence, we checked all animals daily for survival and counted the offspring
163 produced, which were then removed from the beakers. We determined the lifetime fecundity
164 of hosts (the total number of offspring) and host lifespan; both lifespan and fecundity tend to
165 be reduced by *M. bicuspidata* infections (Clay *et al.*, 2019). The dead animals were stored
166 individually in a 1.5 mL tube filled with 100 μL deionized water, and were placed in a
167 refrigerator for subsequent measurement of spore yield, a key component of parasite fitness.
168 Because hosts contain all of the parasite spores that were produced over the course of infection
169 at host death, we quantified spore yield, i.e., the number of spores per host, by grinding dead
170 infected hosts with an electric pellet pestle (Fisher Scientific catalog no.: 12-141-361) for 60
171 seconds. A sample (10 μL) of this solution was added to a Neubauer hemocytometer, and we
172 estimated the spore yield by averaging the number of mature spores and total spores (mature +
173 immature spores) from four grids. Mature spores can be visually distinguished from immature
174 ones (Ebert, 2005; Stewart Merrill *et al.*, 2019); mature ascospores were characterized by their
175 needle shape, each containing a dark band in the center, whereas conidia and needle-shaped
176 spores lacking the central bands and sharp edge were considered immature (see Fig S1 for
177 examples).

178 *Daphnia* that died within 7 days after exposure to parasites were excluded because of early
179 mortality. Any male *Daphnia* that were misidentified as females in the beginning of the
180 experiment were also excluded. This resulted in a total of 141 individuals (current temperature
181 of 20°C: n = 37 and 37 for rearing temperature of 20°C and 24°C, respectively; current
182 temperature of 24°C: n = 35 and 32 for rearing temperature of 20°C and 24°C, respectively).

183

184 *Statistical analyses*

To test for the effects of rearing and current temperature on host fitness, we included rearing temperature (20°C or 24°C) and current temperature (20°C or 24°C) and their interaction as fixed factors. We analyzed the lifetime fecundity using a generalized linear mixed model (GLMM) with a Poisson distribution, and analyzed the survival with a Cox proportional hazard mixed effect model. In both analyses, the parental source of the spores (i.e., the identity of infected host individual) was included as a random effect. We included this random effect because spores were derived from different host individuals; spores from a single individual were used to infect pairs of new hosts (one at 20°C and one at 24°C), with spores from a single individual used to infect 1-3 pairs of hosts.

We analyzed the effects of rearing and current temperature on parasite fitness in a similar fashion by including rearing and current temperature and their interaction as fixed factors. We analyzed the number of mature spores and total number of spores using GLMMs with a Gaussian distribution; we took the natural log of the number of spores plus one prior to analyses to meet the requirements of normality for regressions. We analyzed the probability of infection and the proportion of spore maturation (the number of mature spores divided by the total number of spores) using GLMMs with a binomial distribution and logit link function. Similarly, the parental source of the spores was included as a random effect.

GLMMs were conducted with the glmer function in the *lme4* package (Bates *et al.*, 2015), whereas the Cox proportional hazard mixed effect model was conducted in the *coxme* package (Therneau, 2012). We started the analyses by including the interaction terms, with non-significant interactions removed from the models. If significant interaction terms were detected, pairwise post-hoc comparisons were made to assess differences between individual treatments in the *emmeans* package (Lenth, 2021). All analyses were conducted in R version 4.1.2 (R Development Core Team, 2014).

Results

There was no effect of rearing temperature but a marginally significant effect of current temperature on infection prevalence: infections carried out at warmer temperatures led to a marginally lower infection prevalence than cooler temperatures (χ^2 = 3.29, p = 0.070), regardless of whether the spores were reared at cooler or warmer temperatures (Fig. 1A; χ^2 = 0.13, p = 0.721). Among 141 *D. dentifera* exposed to *M. bicuspidata* spores, 82.4% (61 out of 74) and 70.1% (47 out of 67) developed terminal infection at current cooler and warmer temperatures, respectively.

For hosts that were successfully infected, both current (χ^2 = 9.40, p = 0.002) and rearing (χ^2 = 5.58, p = 0.018) warmer temperatures increased total spore yield (Fig. 1B). Of the spores produced, a smaller proportion became mature when current temperatures were warmer (59.2% at 20°C vs. 36.3% at 24°C; χ^2 = 33.22, p < 0.001) and when parasites were reared at cooler temperatures (46.5% at 20°C vs. 51.9% at 24°C; χ^2 = 6.74, p = 0.009; Fig. 1C). Moreover, current and rearing temperatures interacted in affecting the mature spore yield per host — i.e., the yield of spores that had reached the developmental stage with the potential to initiate a new infection (current temperatures x rearing temperatures: χ^2 = 4.62, p = 0.032; Fig. 1D). Specifically, when infecting hosts at cooler temperatures, parasites that developed at warmer temperatures produced more mature spores compared to those that developed at cooler temperatures (t = -3.30, p = 0.002), yet there was no such difference between spores that developed at cooler versus warmer temperatures when infecting hosts at warmer temperatures (t = -0.14, p = 0.891; Fig. 1D). For parasites that developed at cooler temperatures, spore yield was similar at both cooler and warmer current temperatures (t = -0.30, p = 0.765, compare blue bars in Fig. 1D). However, parasites reared at warmer temperatures produced fewer mature spores at current warmer temperatures compared to current cooler temperatures (t = 2.73, p = 0.008, compare red bars in Fig. 1D).

In contrast to the results for spore yield and maturation, parasite virulence was influenced by current temperature but not rearing temperature (Fig. 2). There was no effect of the temperature at which parasites were reared on the total number of host offspring ($\chi^2 = 1.45$, d.f. = 1, $P = 0.228$). Fecundity was only affected by current temperatures, with fewer host offspring produced at warmer temperatures ($\chi^2 = 10.67$, d.f. = 1, $P = 0.001$; Fig. 2A). Similar patterns were found in survival probability, such that there was no effect of rearing temperatures ($\chi^2 = 1.13$, d.f. = 1, $P = 0.288$) but a significant effect of current temperatures – infected individuals were more likely to die early at warmer temperatures ($\chi^2 = 76.36$, d.f. = 1, $P < 0.001$; Fig. 2B).

Discussion

Transgenerational plasticity has the potential to influence organisms' responses to rapidly changing environments, yet whether transgenerational plasticity of parasites exists, and whether it alters parasite fitness and/or virulence, have been overlooked. By investigating the impacts of temperature in both rearing and current environments, we showed that infection prevalence depended mainly on current temperatures, whereas reproductive success of the parasite was determined by the interplay between the (rearing) temperature at which spores developed and the (current) temperature at which the new infections occurred. Infections by parasites that had been reared at warmer temperatures yielded more mature spores, but only at cooler current temperatures. Thus, our findings demonstrate transgenerational plasticity of parasites in response to changing temperatures but does not provide evidence for adaptive 'priming' of parasites.

We found that the prevalence of infection was explained by offspring infection temperatures but not by rearing temperatures; this suggests that *M. bicuspidata* spores were equally viable, irrespective of previous temperatures. This finding is consistent with previous findings (Little *et al.*, 2007) on *D. magna* and their interactions with the bacterial parasite

Pasteuria ramosa, one of the best-studied parasites of *Daphnia*. A prior study found that current temperatures, rather than parasite rearing temperatures, influenced parasite infectivity, with highest infections at 20°C (Vale *et al.*, 2008). Moreover, spore production per host was also highest at this temperature (Vale *et al.*, 2008). In another study, optimal performance of the microsporidium parasite *Ordospora colligata* showed similar patterns (Kunze *et al.*, 2022), peaking at ~20°C and decreasing at both higher and lower range of temperature gradient. Our results are also in line with previous work on the *Daphnia*-*Metschnikowia* system in that higher current temperatures reduced parasite infectivity (Shocket *et al.*, 2018). However, we did not find the same pattern in the effect of rearing temperatures. The earlier study found that higher rearing temperatures increased infectivity (Shocket *et al.*, 2018), whereas we did not find a significant effect of rearing temperature on infectivity; one possible explanation for this difference is that the earlier study explored rearing temperatures of 15-22°C whereas in our experiment temperature spanned from 20-24°C to simulate a shift towards a higher temperature range. Future work considering a wider range of temperatures would help to uncover whether spores reared at intermediate temperatures are most infectious.

Contrary to a previous study which showed that temperature warming can reduce spore production (Vale *et al.*, 2008), we found that parasites actually had greater total spore yield (inclusive of both mature and immature spores) in response to warmer current temperatures. Parasites also produced more spores when the spores that began the infection had been reared under warmer temperatures. That is, warming induced parasites to be more productive. However, higher current and rearing temperatures had opposite effects on spore maturation (Fig. 1C): warmer current temperatures substantially reduced spore maturation, but warmer rearing temperatures increased spore maturation. The additive temperature effects of rearing and current temperatures on total spore yield per host, and their contrasting effects on the proportion of spores that matured, mean that warmer rearing temperatures enabled parasites to

produce more mature spores than those reared at cooler temperatures, but only in cooler current environments. Yet we found no evidence to suggest that this transgenerational plasticity was adaptive, since the offspring of parasites reared at high temperatures did not have a fitness advantage at current high temperatures (Fig. 1D). These results corroborate previous studies showing the vulnerability of parasites to sustained exposure over generations to high temperatures (Carlson *et al.*, 2017; Schampera *et al.*, 2022). These findings might explain why seasonal epidemics of *M. bicuspidata* tend to erupt when lake temperatures gradually cool down in late summer (Shocket *et al.*, 2018); in these cooling conditions, the transgenerational plasticity we observed would increase parasite fitness.

A potential caveat arises from the fact that the environment a parasite experiences very early in infection can influence parasite fitness (Röder *et al.*, 2008), and this developmental plasticity could be hard to distinguish from transgenerational plasticity, particularly if the parental conditions influence the likelihood of infection. For example, if more spores successfully infected a host, that might have an impact on the final spore yield, though whether it would lead to more spores (because of a larger initial population size within the host) or fewer (because of resource competition or apparent competition via the immune system) is hard to predict; moreover, given that we know that the parasite shows strongly logistic growth within hosts, differences in initial population size might primarily influence when the parasite reaches its maximal abundance within the host, rather than the final density. While it is difficult to predict how developmental plasticity might impact spore yield, our results related to the proportion of spores that matured do not suggest developmental plasticity. Focusing on the warmer current temperature treatment (where there was a larger, but still non-significant, difference between infection levels for the two rearing treatments), spores from the cooler rearing treatment tended towards lower infection levels than those from the warmer rearing treatment (Fig. 1A). However, this lower infection level was associated with a lower proportion

of spore maturation (Fig. 1C). We cannot come up with a plausible explanation for why an early infection environment with fewer individuals would reduce spore maturation, so we suspect this result was not driven by developmental plasticity. However, future investigations of transgenerational plasticity in parasites should be designed in a way that allows for disentangling transgenerational effects from developmental plasticity.

While parasite fitness was explained both by the effects of rearing and current temperatures, parasite virulence was exclusively determined by current temperatures. Specifically, parasites were more virulent (i.e., greater reduction in host fecundity and lifespan; Fig. 2) at higher temperatures, when the total number of spores was also higher (i.e., suggesting stronger within-host competition; Fig. 1B). This is in line with previous theoretical work suggesting that parasite virulence should be higher when intraspecific competition is intense (Lively, 2006). The absence of an effect of rearing temperatures suggests that parasite transgenerational plasticity had negligible influences on parasite virulence in this system. Instead, parasite virulence was mainly determined by the thermal ecology of host-parasite interactions in current environments, consistent with another recent study (Schampera *et al.*, 2022).

Together, our results highlight the importance of incorporating parasite infection history and the potential for context-dependent transgenerational plasticity. Future research should consider how transgenerational plasticity contributes to parasite adaptation and its consequence on disease transmission, in order to better understand host-parasite interactions in changing environments.

Data (heading only required if data is available elsewhere)

The data and code used for this study are available on GitHub (https://github.com/syuanjyunsun/parasite-transgen-exp).

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Author Contributions

SJS, MKD, and MAD conceived the study. SJS, MKD, KMM, and MAD designed the experiments. SJS, MKD, KMM, and RNJ conducted the experiments. SJS performed data analysis. SJS wrote the initial draft of the manuscript and all authors contributed to editing.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Standards

Not applicable.

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Fig. 1. Parasite fitness was affected by both rearing and current temperatures, indicating transgenerational effects of temperature on parasites. Infection prevalence (A) was not as strongly impacted by temperature as was parasite fitness, measured as the number of total spore yield (B), proportion of spore maturation (C), and mature spore yield (D). Means and standard error bars are shown. The box plots show median values, the 25^{th} and 75^{th} percentiles, and interquartile ranges. Significant (*) and non-significant (ns) differences between treatments are indicated on panel D.

Fig. 2. Virulence was affected by current temperature but not rearing temperature. This held both for virulence measured as the total number of offspring (A) and as survival probability (B). The colors indicate temperatures at which parasites were reared. The box plot shows median values, the 25^{th} and 75^{th} percentiles, and interquartile ranges.