Transgenerational plasticity in a zooplankton in response to temperature elevation and parasitism

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Abstract

Organisms are increasingly facing multiple stressors, which can simultaneously interact to cause unpredictable impacts compared to a single stressor alone. Recent evidence suggests that phenotypic plasticity can allow for rapid responses to altered environments, including biotic and abiotic stressors, both within a generation and across generations (transgenerational plasticity). Parents can potentially 'prime' their offspring to better cope with similar stressors, or, alternatively, might produce offspring that are less fit because of energetic constraints. At present, it remains unclear exactly how biotic and abiotic stressors jointly mediate the responses of transgenerational plasticity, and whether this plasticity is adaptive. Here we test the effects of biotic and abiotic environmental changes on within- and trans-generational plasticity using a *Daphnia-Metschnikowia* zooplankton-fungal parasite system. By exposing parents and their offspring consecutively to the single and combined effects of temperature elevation and parasite infection, we showed that transgenerational plasticity induced by temperature and parasite stress influenced host fecundity and lifespan; offspring of mothers that were exposed to one of the stressors were better able to tolerate temperature elevation, compared to offspring of mothers that were exposed to neither or both stressors. Yet the negative effects caused by parasite infection were much stronger, and this greater reduction in host fitness was not mitigated by transgenerational plasticity. We also showed that temperature elevation led to a lower average immune response, but the nature of its relationship with fecundity reversed under elevated temperatures; this suggests that parents that were exposed to parasites can potentially prime their offspring to respond to the joint stressors of both temperature elevation and parasite infection. Together, our results highlight the need to address questions at the interface of multiple stressors and transgenerational plasticity, and the importance of considering multiple fitness-associated traits when evaluating the adaptive value of transgenerational plasticity under changing environments.

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The dataset and R scripts are openly available in GitHub (https://github.com/syuanjyunsun/host_trans_plasticity).

Abstract

Organisms are increasingly facing multiple stressors, which can simultaneously interact to cause unpredictable impacts compared to a single stressor alone. Recent evidence suggests that phenotypic plasticity can allow for rapid responses to altered environments, including biotic and abiotic stressors, both within a generation and across generations (transgenerational plasticity). Parents can potentially 'prime' their offspring to better cope with similar stressors, or, alternatively, might produce offspring that are less fit because of energetic constraints. At present, it remains unclear exactly how biotic and abiotic stressors jointly mediate the responses of transgenerational plasticity, and whether this plasticity is adaptive. Here we test the effects of biotic and abiotic environmental changes on within- and trans-generational plasticity using a *Daphnia-Metschnikowia* zooplankton-fungal parasite system. By exposing parents and their offspring consecutively to the single and combined effects of temperature elevation and parasite infection, we showed that transgenerational plasticity induced by temperature and parasite stress influenced host fecundity and lifespan; offspring of mothers that were exposed to one of the stressors were better able to tolerate temperature elevation, compared to offspring of mothers that were exposed to neither or both stressors. Yet the negative effects caused by parasite infection were much stronger, and this greater reduction in host fitness was not mitigated by transgenerational plasticity. We also showed that temperature elevation led to a lower average immune response, but the nature of its relationship with fecundity reversed under elevated temperatures; this suggests that parents that were exposed to parasites can potentially prime their offspring to respond to the joint stressors of both temperature elevation and parasite infection. Together, our results highlight the need to address questions at the interface of multiple stressors and transgenerational plasticity, and the importance of considering multiple fitness-associated traits when evaluating the adaptive value of transgenerational plasticity under changing environments.

Introduction

Understanding how populations and species respond to altered environments is critical in a rapidly changing world (de Laender *et al.* 2016; García *et al.* 2018). Adaptation can help organisms cope with environmental changes (Fox *et al.* 2019), but can require relatively long time scales that may not allow species to keep up with the pace of change (Visser 2008; Radchuk *et al.* 2019). Fortunately, phenotypic plasticity can allow organisms to weather the negative impacts of changing environments on shorter time scales (Snell-Rood *et al.* 2018), with studies of single stressors showing that phenotypic plasticity can increase fitness in changing environments and even facilitate rapid adaptation (Levis & Pfennig 2016; Chevin & Hoffmann 2017; Sun *et al.* 2020). Phenotypic plasticity can not only influence responses within generations, but also across generations (i.e., transgenerational plasticity or maternal effects). Transgenerational plasticity is particularly important for offspring to buffer the adverse impacts of the immediate environment, especially when the environmental cues experienced by previous generations match those of the offspring generation (Mousseau & Fox 1998). In short, transgenerational plasticity has the potential to allow organisms to cope with the same or different stressors across generations (Tran *et al.* 2019; Meng *et al.* 2021).

Environmental stressors, such as temperature increase, land use change, and toxicants, often occur simultaneously and can interact in complex and unpredictable ways (Schäfer & Piggott 2018; Jackson *et al.* 2021; Simmons *et al.* 2021). A growing body of work in multiple-stressor research has focused on understanding and predicting interactions between different stressors, which can cause antagonistic or synergistic effects compared to an individual stressor (Orr *et al.* 2020). Moreover, these responses can occur across generations, with the potential for parents to 'prime' their offspring to better handle stressful environments (Tran *et al.* 2019). While it is clear that transgenerational plasticity can impact offspring fitness in the face of multiple stressors, to date studies have focused primarily on abiotic stressors. This is an important limitation because the shifts in abiotic conditions that are common under global climate change routinely occur alongside changes in biotic factors (e.g., parasites and predators).

A long-standing idea is that climate warming may exacerbate the negative effects of parasites, partly because elevated temperatures increase the fitness of the parasites and/or weaken host defenses (Harvell *et al.* 2002). However, studies of multiple stressors show that it can be challenging to predict whether a combination of stressors will increase or decrease the impact of a given stressor (Piggott *et al.* 2015; Orr *et al.* 2020). In aquatic species, for example, warming can increase the toxicity of several pesticides (Noyes *et al.* 2009; Moe *et al.* 2013) but, in other cases, can decrease pesticide toxicity due to more rapid degradation (op de Beeck *et al.* 2017). Moreover, studies of the joint effects of elevated temperature and parasitism have generally overlooked the possibility that transgenerational effects might alter the impact of these stressors. Host parents who are challenged by parasites can potentially enhance the immune responses of offspring generation when challenged by the same parasites, a type of transgenerational plasticity also known as 'transgenerational immune priming'

(Sadd *et al.* 2005; Tetreau *et al.* 2019). However, while it is clear that multiple stressors can interact with one another, and that transgenerational plasticity can impact offspring fitness in the face of stressors, most studies of transgenerational plasticity to date have focused on single biotic or abiotic factors (but see (Roth & Landis 2017)), leaving a major gap in understanding transgenerational effects in the context of multiple-stressor research.

Transgenerational plasticity in the face of multiple stressors might increase offspring fitness, especially when the two stressors involve similar physiological mechanisms and when they are predictable. In contrast, two distinct forms of stressors may hinder the adaptive value of transgenerational plasticity not only because the reduced likelihood that multiple environmental variables match across generations, but also because protecting against one stressor might increase vulnerability to another; for example, shifts in temperatures in combination with induced pathogen prevalence elevated the energetic costs that are required for acclimation (Roth & Landis 2017).

In this study, we tested for within- and trans-generational effects of abiotic and biotic environmental changes, namely temperature elevation and parasite infection, on host performance using a *Daphnia-Metschnikowia* zooplankton-fungal parasite system. Specifically, we examined the single and combined effects of mean temperature elevation and parasite infection in the parental generation and investigated their offspring's response to the single and combined effects of temperature elevation and parasite infection. We hypothesized that parents should produce offspring that are primed to live in similar environments, and thus perform better than unprimed offspring (the "environmental matching hypothesis"). Alternatively, parents challenged with stressful environments might have less fit offspring, regardless of the type of stressor, due to reduced resources for reproduction (the "stress hypothesis"). Furthermore, we hypothesized that temperature elevation and parasite infection of parents would have an interactive effect on offspring performance.

Material and Methods

Study system

We focused on the crustacean *Daphnia dentifera*, which is commonly found in stratified lakes in Midwestern Northern America (Tessier *et al.* 1263). Lakes in this temperature region have increased in temperature by 0.5-1.0°C relative to 1951-1980 (Piccolroaz *et al.* 2020), with further increases expected, including a 3 to 25x increased likelihood of severe lake heatwaves with 1.5-3.5°C warming (Woolway *et al.* 2022). *D. dentifera* are exposed to the fungal parasite *Metschnikowia bicuspidata* during filter-feeding for algal food, with epidemics typically beginning during late summer/early fall (Shocket *et al.* 2019). *M. bicuspidata* virulently reduces host fecundity and lifespan (Clay *et al.* 2019).

Experimental setup

Assessing the adaptive significance of transgenerational plasticity in response to the single or combined effects of environmental stressors requires a fully factorial design manipulating each of stressors in both parental and offspring generations (Donelson *et al.* 2018). This approach allows the fitness components to be fully dissected to evaluate

the adaptive value of within- and trans-generational effects when parental and offspring environments are matched or mismatched.

To test for within- and trans-generational effects of temperature elevation and/or parasite infection, we conducted a fully factorial experiment over two generations (Fig. 1). This experiment used the “Standard” lab lines of *D. dentifera* and *M. bicuspidata* originally isolated from a lake in Barry County, Michigan. We describe the maintenance of the *D. dentifera* and *M. bicuspidata* used in this study in more detail elsewhere (Sun *et al.* 2022a). Immediately prior to this experiment, *D. dentifera* were maintained in standardized conditions (a 16:8 photoperiod at 22°C) for three generations and fed three times a week with a phytoplankton food (*Ankistrodesmus falcatus*, 20,000 cells/mL). *M. bicuspidata* spores (2 weeks-1 month old) were harvested from *D. dentifera* previously infected by *M. bicuspidata* at an exposure density of 250 spores/mL. Infected *D. dentifera* were stored in a refrigerator before use and were ground up prior to exposure using a cordless pellet pestle (Fisherbrand, Fisher Scientific).

In the parental generation (F0), *Daphnia* were exposed to one of the four treatment combinations that factorially combined temperature elevation (20°C and 24°C) and parasite exposure (control/exposed). We collected neonates from the second clutch of the acclimated *D. dentifera* stock populations on the day of birth and placed them either at 20°C or 24°C. Each animal was kept individually in a 50 mL beaker filled with 50 mL lake water and fed three times a week (20,000 cells/mL *A. falcatus*). For the parasite exposure treatment, we added *M. bicuspidata* spores at a density of 145 spores/mL to each beaker when juveniles were 6 days and 5 days old for 20°C and 24°C, respectively. This degree-day approach allows for the same accumulated product of time and temperature at degree-day 120 (Vale *et al.* 2008; Manzi *et al.* 2020), thus minimizing potential differences in body size between temperature treatments (as confirmed statistically: $\chi^2 = 2.19$, d.f. = 1, $P = 0.139$). For the unexposed animals, a placebo solution containing the same amount of dead uninfected *D. dentifera* was added to each beaker. The animals were exposed to either the parasite or placebo solution for 24 hours, fed 20,000 cells/mL *A. falcatus*, and kept at 16:8 light:dark cycle. All experimental animals were then transferred to new beakers filled with 50 mL spore-free lake water, fed 20,000 cells/mL *A. falcatus*, and maintained at 16:8 light:dark until the end of the experiment. To test for within- and trans-generational plasticity in the offspring generation (F1), we collected neonates from the second and third clutches of F0 adults. We used a split brood design in which four neonates from a single brood were haphazardly selected and one individual assigned to each of the four treatment combinations (two temperature treatments (20°C and 24°C) and two parasite exposure treatments (control/exposed)). The experiment was conducted in the same manner in the offspring generation as in the parental generation, and the degree-day approach once again led to similar body size between temperature treatments ($\chi^2 = 0.79$, d.f. = 1, $P = 0.375$). In total, there were 16 different treatment combinations (Fig. 1).

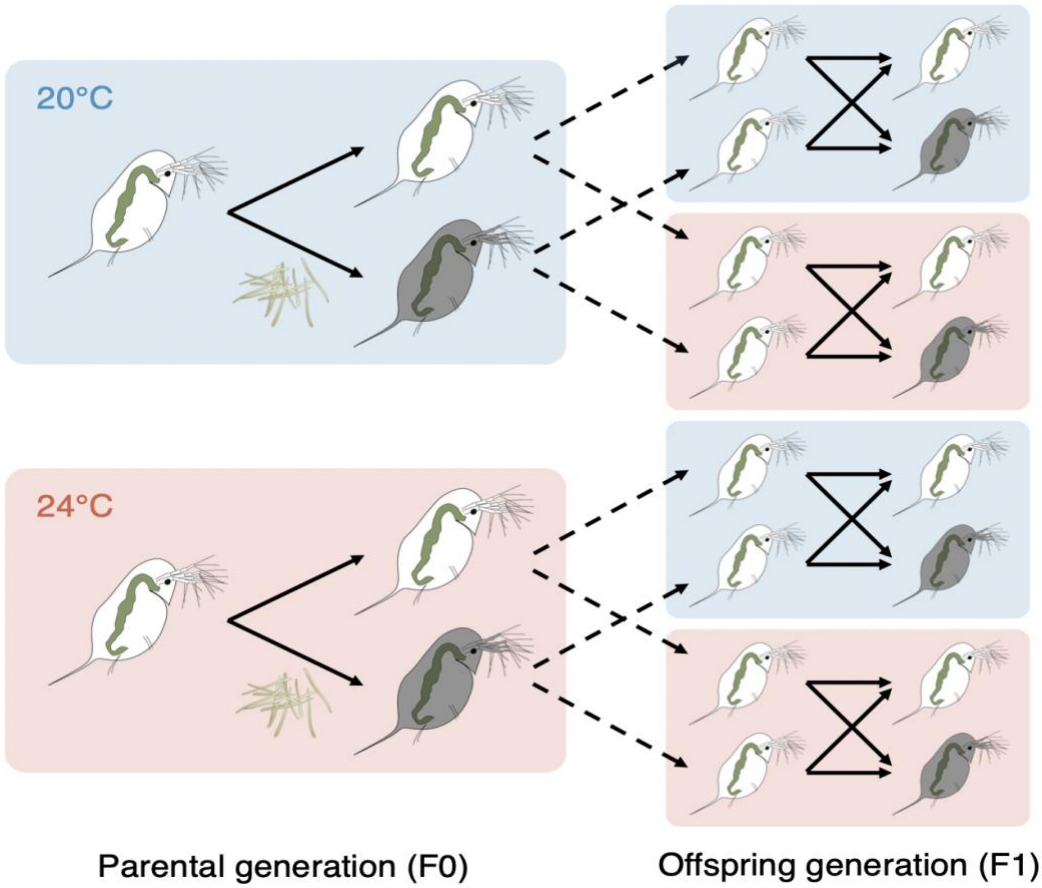

Figure 1. Experimental design used to evaluate whether the single and combined effects of temperature and parasite infection experienced during parental generations (F0) influenced the performance of offspring (F1), and whether this effect depended on the environment of the offspring. Blue shading indicates ambient temperature (20 °C) and red shading indicates elevated temperature (24 °C). Solid lines indicate individuals from a given generation being divided between parasite exposure (gray *D. dentifera*) or placebo exposure (white *D. dentifera*). Dashed lines indicate offspring collected from the F0 generation that were used for the F1 generation treatments.

This experiment relates to, but differs from, two other recent experiments. In the first (Sun *et al.* 2022a), we focused on how temperature modified trait-mediated infection outcomes in the F0 generation and did not look across generations. In the second related experiment (Sun *et al.* 2022b), we looked for evidence of transgenerational plasticity in the parasite (rather than in the host, which is the focus of the present study).

Data collection

To quantify host responses to the parasite at the earliest stages of infection, we examined animals exposed to parasites at the end of the 24 hours inoculation period under an Olympus BX53F compound microscope (200-400X magnification). We scanned the anterior and posterior of the gut, where spores are most likely found penetrating into the host's body cavity (Stewart Merrill *et al.* 2019). We counted the number of spores, categorized into two categories (*sensu* (Stewart Merrill *et al.* 2019)): embedded spores (i.e., partially embedded in the gut epithelium) and hemocoel spores (i.e., penetrated into the body cavity); this allows us to quantify gut resistance (i.e., the extent to which the gut epithelium acts as a barrier to infecting spores) as the number of embedded spores divided by the total number of attacking spores (embedded spores + hemocoel spores). In addition, to quantify the immune response, we counted the total number of hemocytes attaching to the hemocoel spores and determined the number of hemocytes per spore (total number of hemocytes divided by the number of hemocoel spores). At this point, we also determined host body size by measuring the distance between the center of the eye and the base of the tail spine (cellSens Software, Olympus, version 1.18).

To determine host fitness, we checked all animals daily for mortality and counted the number of offspring produced, which were then removed from the beakers. Once the last infected individual was found dead, the unexposed animals were checked twice a week, since uninfected *Daphnia* live significantly longer than infected ones (Sun *et al.* 2022a). Dead infected animals were kept individually in a 1.5 mL tube of 100 µL deionized water and stored in a refrigerator before determining spore yield. We calculated two key components of parasite fitness: proportion of terminal infections (that is, infections that yield transmission spores capable of infecting a new host) and spore yield per infected host (that is, the number of mature transmission spores per host). We determined the spore yield by grinding the host using a cordless pellet pestle (Fisherbrand, Fisher Scientific) for 60 seconds to release spores and homogenize the solution, then adding a 10 µL sample to a Neubauer hemocytometer. We averaged the number of mature spores from four grids for an estimation of spore yield.

Animals that died within 7 days after exposure were excluded from the analysis because of difficulty in determining infection status. We also excluded males, which occurred at relatively low frequency (45 out of 420 total animals).

Data analysis

All analyses were performed in R (version 4.1.2) (R Development Core Team 2014) using generalized linear mixed models (GLMM) with the glmer function in the *lme4* package (Bates *et al.* 2015). Analysis of variance (ANOVA) was performed in the *car*

package (Fox *et al.* 2021). Additional packages used include the *coxme* package (Therneau 2012) for survival analyses, and the *emmeans* package (Lenth 2021) for Tukey *post-hoc* comparisons once significant interaction terms were detected.

In most analyses, we included temperature (F0 Temperature) and parasite exposure (F0 Parasite) of the parental generation, and those of the offspring generation (F1 Temperature and F1 Parasite), as well as the interaction between the four variables (that is, F0 Temperature, F0 Parasite, F1 Temperature, F1 Parasite); exceptions to this are described below. In addition, parent ID was included as a random factor when analyzing data of offspring generation since multiple offspring of the same clutch were used from the same mother.

We were interested in six host traits: two related to resistance to infection (gut resistance and hemocytes per spore), three related to host reproduction (age at first reproduction, first clutch size, and lifetime fecundity), and host survival. We analyzed gut resistance (embedded spores divided by attacking spores, as described above) and hemocytes per spore (after ln(x+1) transformation) with a Gaussian distribution. When analyzing gut resistance, we also included gut epithelium thickness as a covariate. These analyses of resistance to infection included all animals, including those that were exposed to spores but that did not develop terminal infections. For the remaining analyses, we only used unexposed (and, therefore, uninfected) animals and animals that were infected, excluding individuals that were exposed but uninfected. We analyzed age at first reproduction and first clutch size with a Poisson distribution, and lifetime fecundity with a negative binomial distribution to account for overdispersion. However, we note that we didn't expect a within-generation effect of parasite exposure on age at first reproduction or first clutch size, as the experimental animals likely deposited their first clutch in the brood chamber right around the time of parasite exposure; therefore, the results for age at first reproduction and first clutch size are presented in the supplementary information (Figure S1). For the survival analysis, we analyzed host survival with a Cox proportional hazard mixed effect model.

We were also interested in the potential for a trade-off between reproductive success and immune responses. Specifically, we were interested in whether a greater immune response (quantified as hemocytes per spore) would come at a cost of lifetime host reproduction. We were also interested in whether this relationship would be impacted by within- or trans-generational impacts of temperature elevation or parasite exposure. Therefore, this analysis included gut resistance and hemocytes per spore as covariates, in addition to the fixed effects of temperature of both parental and offspring generations (F0 and F1 Temperature) and parasite exposure of the parental generation (F0 Parasite); parasite exposure in the F1 generation was not included because all the individuals in this analysis were exposed to (and infected by) parasites in the F1 generation.

Finally, we were also interested in two key components of parasite fitness: the probability of terminal infection and spore yield per host. For terminal infection outcomes, we analyzed the probability of terminal infection (terminal infection: 1; no

terminal infection: 0) with a binomial distribution and logit link function. Among animals that reached terminal infection, we analyzed the spore yield per host [ln(x+1)] with a Gaussian distribution, and included gut resistance and hemocytes per spore as covariates.

Results

Within- and trans-generational effects of stressors on host fecundity and survival

We detected within- and trans-generational effects of temperature elevation and parasite infection on lifetime fecundity, as evidenced by a significant interactive effect between parental and offspring environment for both temperature elevation and parasite infection (Figure 2A; Table S1). The transgenerational impacts were most pronounced when offspring were not exposed to parasites ('control' bars in Figure 2A). If parents experienced neither stressor (left panel of Figure 2A) or both stressors (right panel of Figure 2A), offspring that were exposed to elevated temperatures suffered lower fecundity as compared to those that were raised at ambient temperatures (neither parental stressor: $z = 2.78$, $p = 0.028$; both parental stressors: $z = 4.88$, $p < 0.001$). In contrast, if the parents were only exposed to one stressor (either parasite exposure, as in the second panel of Figure 2A, or elevated temperatures, as in the third panel of Figure 2A), offspring that were exposed to elevated temperatures had the same fecundity as those raised at ambient temperatures (parents exposed to parasites: $z = 0.92$, $p = 0.795$; parents exposed to elevated temperatures: $z = 1.84$, $p = 0.253$). Overall, these results suggest that a moderate amount of parental stress helped offspring maintain high fecundity in the face of temperature elevation, but high parental stress led to reduced offspring fitness at elevated temperatures. The pattern for offspring exposed to parasites was much simpler: reproduction of infected offspring was consistently low across all parental environments (control/20°C: $z = -2.11$, $p = 0.149$; exposed/20°C: $z = 0.61$, $p = 0.929$; control/24°C: $z = 1.49$, $p = 0.446$; exposed/24°C: $z = 2.19$, $p = 0.125$; 'infected' bars in Figure 2A).

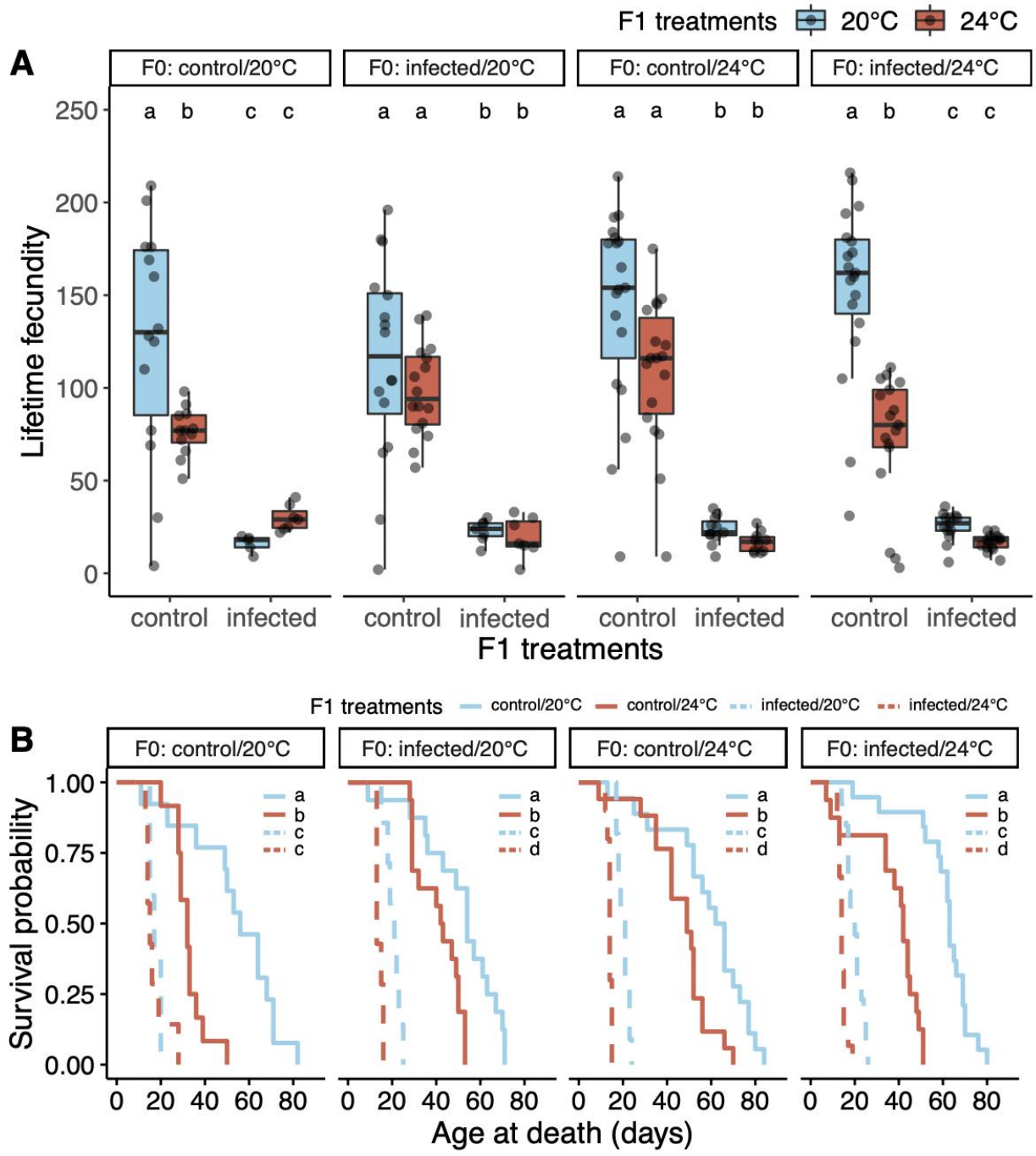

Figure 2. Within- and trans-generational effects of temperature elevation and parasite infection on host fecundity (**A**) and lifespan (**B**). Kaplan-Meier plots in (**B**) show host survival over a period of 84 days. The letters indicate statistically significant differences between treatments in the pairwise comparisons. "F0" = parental generation, "F1" = offspring generation. The box plots show median values, the 25th and 75th percentiles, and interquartile ranges.

Lifespan was also influenced by both parental and offspring environments (Figure 2B; Table S1). For offspring that were not exposed to parasites (solid lines in Figure 2B),

temperature elevation shortened lifespan (red solid lines are to the left of blue solid lines in Figure 2B), but the extent of reduction was greater when their parents were reared under ambient temperatures without parasite infection (z = -5.59, p < 0.001; left panel in Figure 2B) or when parents were exposed simultaneously to temperature elevation and parasite infection (z = -5.26, p < 0.001; right panel in Figure 2B). While elevated temperatures also reduced the survival of unexposed individuals whose parents were exposed to temperature elevation but not parasites (z = -3.61, p = 0.002) or to parasite infection but not elevated temperature (z = -3.50, p = 0.003), this reduction was more modest (that is, the solid red lines on the two center panels in Figure 2B are not as far from the blue lines, as compared to the left and right panels). Furthermore, comparing the differences in lifespan of offspring exposed to temperature elevation alone, individuals whose parents were exposed singly to temperature elevation had higher survival probability compared to those exposed to both temperature elevation and parasite infection (z = -2.69, p = 0.036), and to those never exposed to any of these stressors before (z = 3.86, p < 0.001). Offspring infected by parasites (dashed lines in Figure 2B) died earlier than uninfected hosts (solid lines), with a greater lifespan reduction at elevated than ambient temperatures when parents were exposed to stressful environments (exposed/20°C: z = -3.33, p = 0.005; control/24°C: z = -3.97, p < 0.001; exposed/24°C: z = -4.17, p < 0.001), although no difference was found when parents were unexposed to any stressor (z = 0.37, p = 0.983).

Overall, when offspring were not exposed to parasites, offspring of mothers who were exposed to neither stressor or to both stressors suffered the most when exposed to elevated temperatures, with reduced lifetime fecundity and shorter lifespans; in contrast, elevated temperature had more modest impacts on the unexposed offspring of mothers who experienced only one of the two stressors. For offspring that were infected by the parasite, all individuals suffered strong and consistent reductions in fecundity and similar reductions in lifespan regardless of maternal environment and current temperature.

Within- and trans-generational effects on host immune responses

Gut resistance to attacking spores was similar across all parental and offspring treatments (Figure S2A; Table S1). In contrast, the number of hemocytes per spore was determined by temperature in offspring generations (Figure S2B; Table S1). Specifically, temperature elevation consistently led to fewer hemocytes per spore in offspring generations.

Potential trade-off between immune response and host reproduction

Immune responses were correlated with lifetime fecundity, but in opposite directions at ambient vs. elevated temperatures (Figure 3; Table S3). At ambient temperatures, there is evidence of a trade-off between investment in immune responses and reproduction: individuals that mobilized more hemocytes per spore had lower lifetime fecundity, both for offspring of parents that had been exposed to parasites (χ^2 = 5.78, d.f. = 1, p = 0.016; Figure 3A, blue line) and of parents that had not been exposed to parasites (χ^2 = 9.05, d.f. = 1, p = 0.003; Figure 3B, blue line). In contrast, at elevated temperatures, there was no significant relationship between immune response and fecundity for

offspring of parents that had not been exposed to parasites (unexposed: $\chi^2 = 0.27$, d.f. = 1, $p = 0.602$, Figure 3A red line), and, for offspring of parents that had been exposed to parasites, the pattern reversed: individuals that mobilized more hemocytes per spore had higher lifetime fecundity (exposed: $\chi^2 = 1.99$, d.f. = 1, $p = 0.047$, Figure 3B red line).

Within- and trans-generational effects on terminal infection and spore yield

Temperature treatments did not influence the probability of terminal infection. Parental environment also did not influence the probability of terminal infection (Figure 4A; Table S2). For hosts that developed terminal infection, the spore yield per host was lower at elevated temperatures (Figure 4B; Table S2); neither temperature nor parasite treatments during the parental generation had an effect.

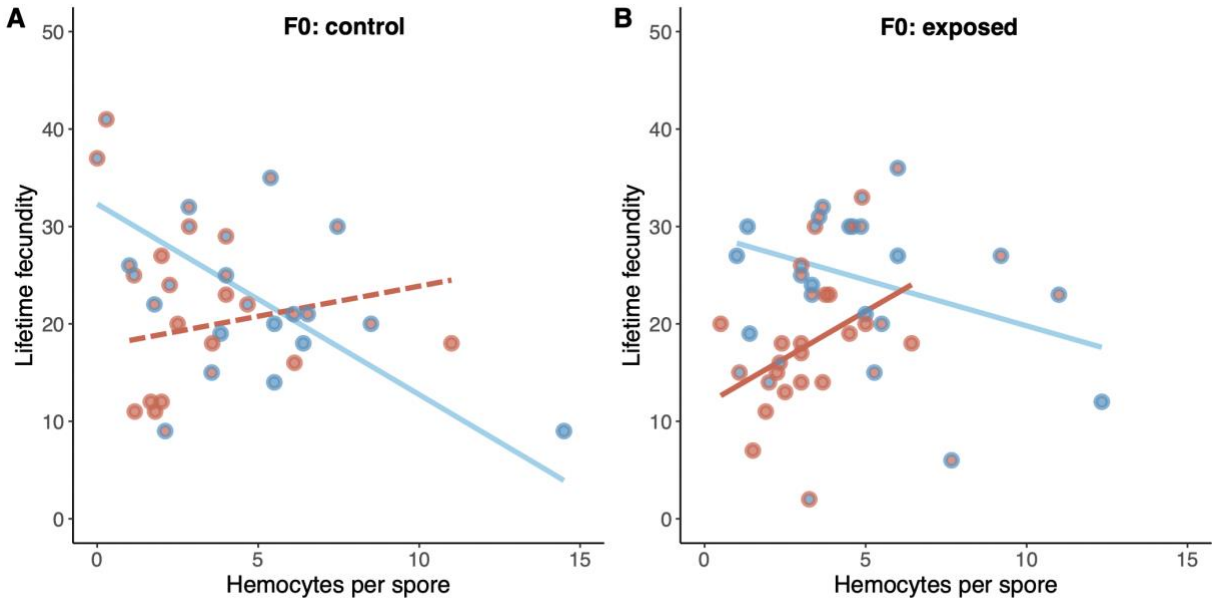

Figure 3. Within- and trans-generational effects of temperature elevation on the relationship between lifetime fecundity and hemocytes per spore in the offspring generation whose parental generations were unexposed (**A**) or exposed (**B**) to parasites. Solid and dashed lines represent significant and non-significant relationships predicted from GLMMs, respectively. The overall model was (reproduction~F0parasite*(F1temp+F0temp)*(Hemocytes.by.spore)+F1temp*F0temp+gut.resistance+(1|source)); both the F0parasite*F0temp*(Hemocytes.by.spore) and F0parasite*F1temp*(Hemocytes.by.spore) interactions were significant (Table S3). Because both parental (F0) and offspring (F1) temperatures influenced reproduction, fill colors denote temperature treatments of the parental generation (blue fills are for 20°C; red fills are for 24°C), and the outline colors denote temperature treatments of the offspring generation (blue outlines are for 20°C; red outlines are for 24°C). In both panels, the regression lines are grouped according to the results of the model; in A, the regression lines are divided according to parental generation temperature (20°C F0 blue line, 24°C F0 red line), whereas in B, the regression lines are divided according to offspring (F1) temperatures.

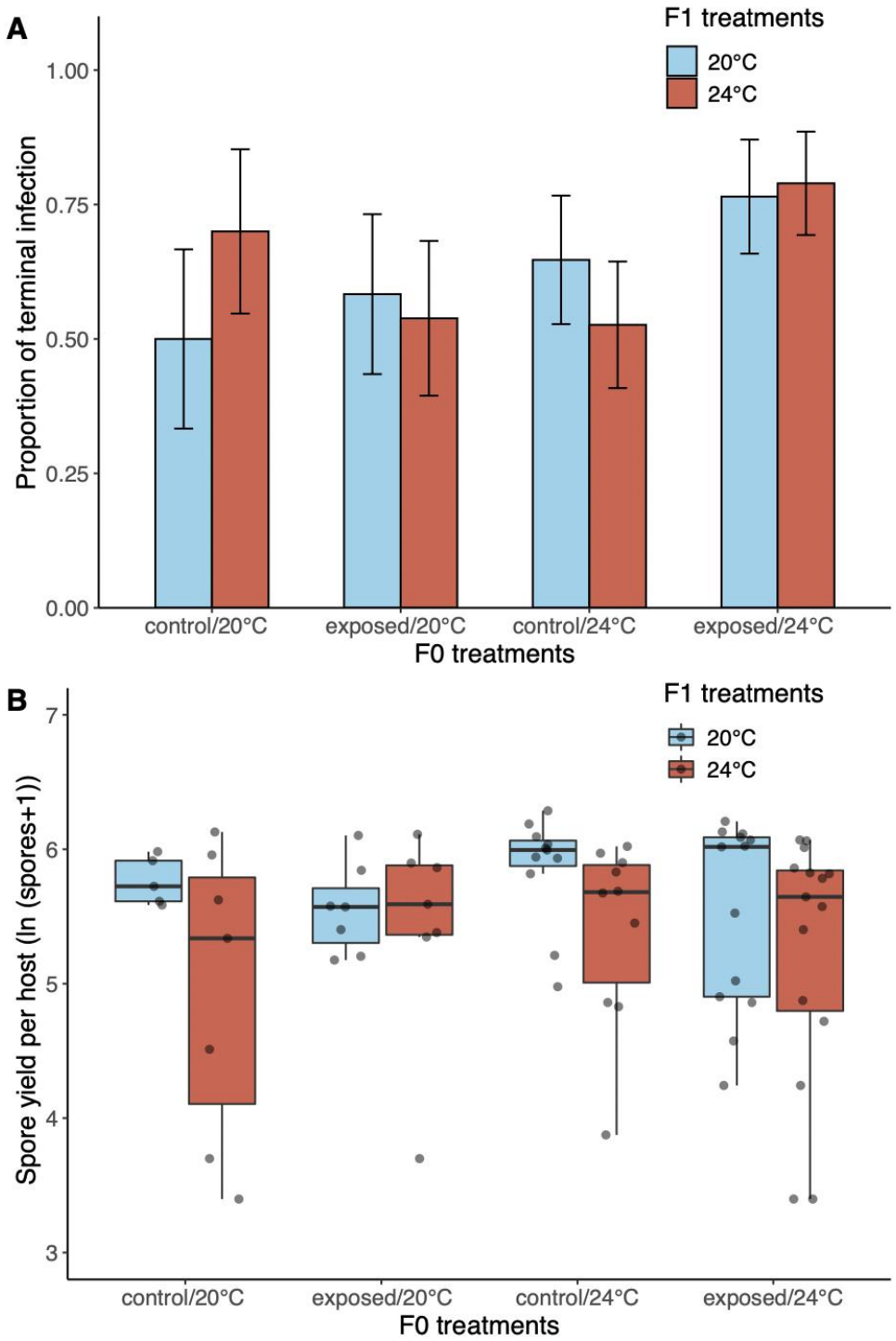

Figure 4. Within- and trans-generational effects of temperature elevation and parasite infection on the probability of terminal infection (**A**) and spore yield per host (ln (spores+1)) (**B**). Means and standard error bars are shown. "F0" = parental generation, "F1" = offspring generation. The box plots show median values, the 25th and 75th percentiles, and interquartile ranges.

Discussion

Transgenerational plasticity can allow organisms to respond rapidly to changing environments, potentially protecting them from fitness loss associated with stressors (Uller 2008; S & SB 2012; Donelson *et al.* 2018). Yet, the ability of transgenerational plasticity to counteract the joint influence of biotic and abiotic stressors has been understudied, limiting our understanding of the role of transgenerational plasticity in a variable world. Here, we found that transgenerational plasticity induced by temperature and parasite stress influenced host performance. This effect was particularly prominent for offspring that were exposed to temperature stress but not parasitism: in this case, offspring of mothers that were exposed to one stressor (either temperature or parasite stress) were better able to tolerate elevated temperatures, as compared to offspring of mothers who experienced neither or both stressors. However, parasite stress had much stronger negative effects on host fitness than temperature stress did, and the large reduction in host fitness arising from infection was not mitigated by transgenerational plasticity. Thus, transgenerational plasticity helped offspring maintain fitness in the face of elevated temperatures if the parents had experienced only one stressor, but did not protect offspring exposed to parasites. In contrast, parasite fitness was mostly unaffected by host transgenerational plasticity. Together, our results provide evidence of transgenerational plasticity, but the degree to which it benefitted the host depended on the identity and combination of environmental stressors.

Our results partially supported the environmental matching hypothesis (Paraskevopoulou *et al.* 2022), wherein parents prime their offspring to better deal with stressors. In our study, elevated temperatures represented stressful environments, reducing fecundity and lifespan. However, offspring of parents who experienced elevated temperatures suffered less (in terms of fecundity and lifespan) than did offspring of parents who experienced ambient temperatures. This finding differs from a finding on a different *Daphnia*-parasite system (Hector *et al.* 2021), which found little effect of maternal temperature. Interestingly, offspring of parents exposed to parasites also suffered less at elevated temperatures compared to ambient temperatures. One possible explanation for this is the potential for shared physiological responses to parasite exposure and temperature stress. Heat-shock proteins, which maintain cellular stability and resistance to heat (Zhang *et al.* 2014). While named after their role in responding to heat stress, heat shock proteins can be upregulated by a wide variety of stressors, including parasite exposure (Selbach *et al.* 2020). Upregulated physiological responses to heat stress in response to parasite infection are common in many taxa, including fish, birds, and mammals (Forsyth *et al.* 1997; Merino *et al.* 1998; Martinez *et al.* 1999). However, offspring of parents that were simultaneously exposed to temperature and parasite stressors suffered the full negative impacts of elevated temperatures. Together, these results suggest that transgenerational effects can help organisms cope with changing environmental conditions, and that previous exposure to biotic and abiotic stressors can both facilitate adaptation to abiotic stressors. Yet, our results also suggest there may be a limit to the ability of transgenerational plasticity to protect offspring in more stressful environments, possibly because resources, which must be allocated simultaneously to both biotic and abiotic stressors, are limited (Bubliy *et al.* 2012).

Beyond the finding that all infected hosts suffered large reductions in fecundity and lifespan (Fig. 2), as expected given the known virulence of this parasite, two other patterns stand out. First, temperature elevation led to a lower immune response, on average, with fewer hemocytes recruited per penetrated spore (Fig. S2B). Second, the nature of the relationship between immune responses and host fecundity reversed under elevated temperatures (Fig. 3). We hypothesized that there might be a trade-off between fecundity and immune responses, as has been seen in many other systems (Gwynn *et al.* 2005; Schwenke *et al.* 2016); such a tradeoff could arise if mounting a strong immune response prevents hosts from investing as many resources in reproduction. At ambient temperatures, a stronger immune response was indeed associated with lower reproductive success, irrespective of parental exposure to parasites (Fig. 3). Surprisingly, this tradeoff disappeared under temperature elevation: the fecundity-immune response relationship was flattened when the parental generation experienced temperature elevation but was not exposed to parasites (Fig. 3A) and became positive when offspring encountered temperature elevation and when parents had been exposed to parasites (Figure 3B). This suggests that parents that were exposed to parasites can potentially prime offspring generations to face the joint stressors of both temperature elevation and parasite infection. The exact mechanism of such immune priming effect has yet to be investigated, but might occur via epigenetic inheritance (Curley *et al.* 2011). These findings highlight the importance of considering transgenerational effects in response to different environmental challenges when exploring trade-offs, and the importance of incorporating multiple fitness components to evaluate the adaptive value of transgenerational effects.

Although physical and immune responses are two potent defenses against parasite infection, we instead found that neither gut resistance nor hemocytes per spore explain differences in spore yield per host. Temperature elevation also had negligible effects on the probability of infection and spore production for hosts that were infected, except that infected hosts generally produced fewer spores when the offspring generation was exposed to elevated temperatures. These findings, alongside the effects of temperature on hosts, suggest that temperature elevation and parasites mainly acted independently in affecting host's fitness components, but temperature can indirectly alter the direction of the fecundity-immune response relationship via within- and trans-generational effects (Fig. S2).

Our results show that transgenerational plasticity helped individuals cope with an abiotic stressor. However, this only occurred when parents were moderately stressed (by either the abiotic or the biotic stressor). Offspring of parents simultaneously exposed to both abiotic and biotic stressors suffered large fitness reductions when exposed to the abiotic stressor, potentially revealing a limit of adaptive transgenerational plasticity. Moreover, the identity of the stressor clearly matters: transgenerational plasticity did not protect individuals that were exposed to the biotic stressor. Furthermore, our results demonstrate the importance of considering multiple fitness-associated traits to understand the adaptive values of transgenerational plasticity induced by multiple stressors in a changing world: adaptive transgenerational plasticity might be masked

without a complete screening of key traits involving performance trade-offs. Future studies identifying the molecular mechanisms, e.g., epigenetic modifications, at various stages of ontogeny (Donelan *et al.* 2020) would be particularly valuable in order to help improve our understanding of the role of transgenerational plasticity in a rapidly changing world.

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Supplementary information

Supplementary results related to host reproduction

Age at first reproduction

We observed within- and trans-generational impacts of elevated temperatures on age at first reproduction. Both parental and offspring temperature impacted age at first reproduction (Fig. S1A; Table S1). The earliest reproduction was by individuals raised at 24°C whose mothers had also been raised at 24°C, while the latest first reproduction was by individuals raised at 20°C whose mothers had also been raised at 20°C. In contrast to the temperature results, parasite exposure in the parental or offspring generation did not significantly impact age at first reproduction (Fig. S1A; Table S1).

First clutch size

Neither temperature elevation nor parasite infection in the parental or offspring generation significantly impacted first clutch size. Instead, first clutch size was similar across all parental and offspring treatments (Fig. S1B; Table S1).

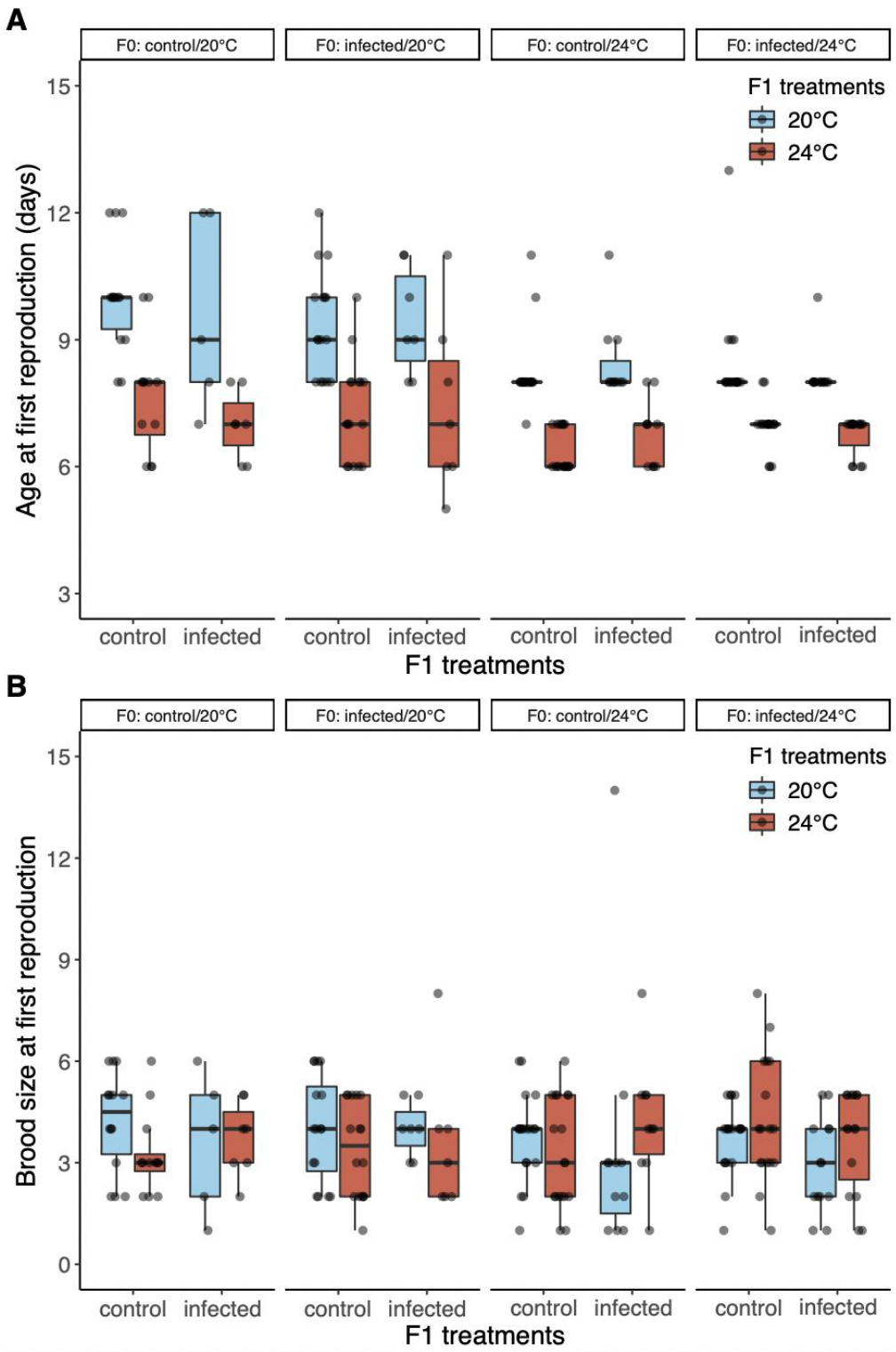

Figure S1. Within- and trans-generational effects of temperature elevation and parasite infection on host age at first reproduction (**A**) and brood size at first reproduction (**B**). "F0" = parental generation, "F1" = offspring generation. The box plots show median values, the 25th and 75th percentiles, and interquartile ranges.

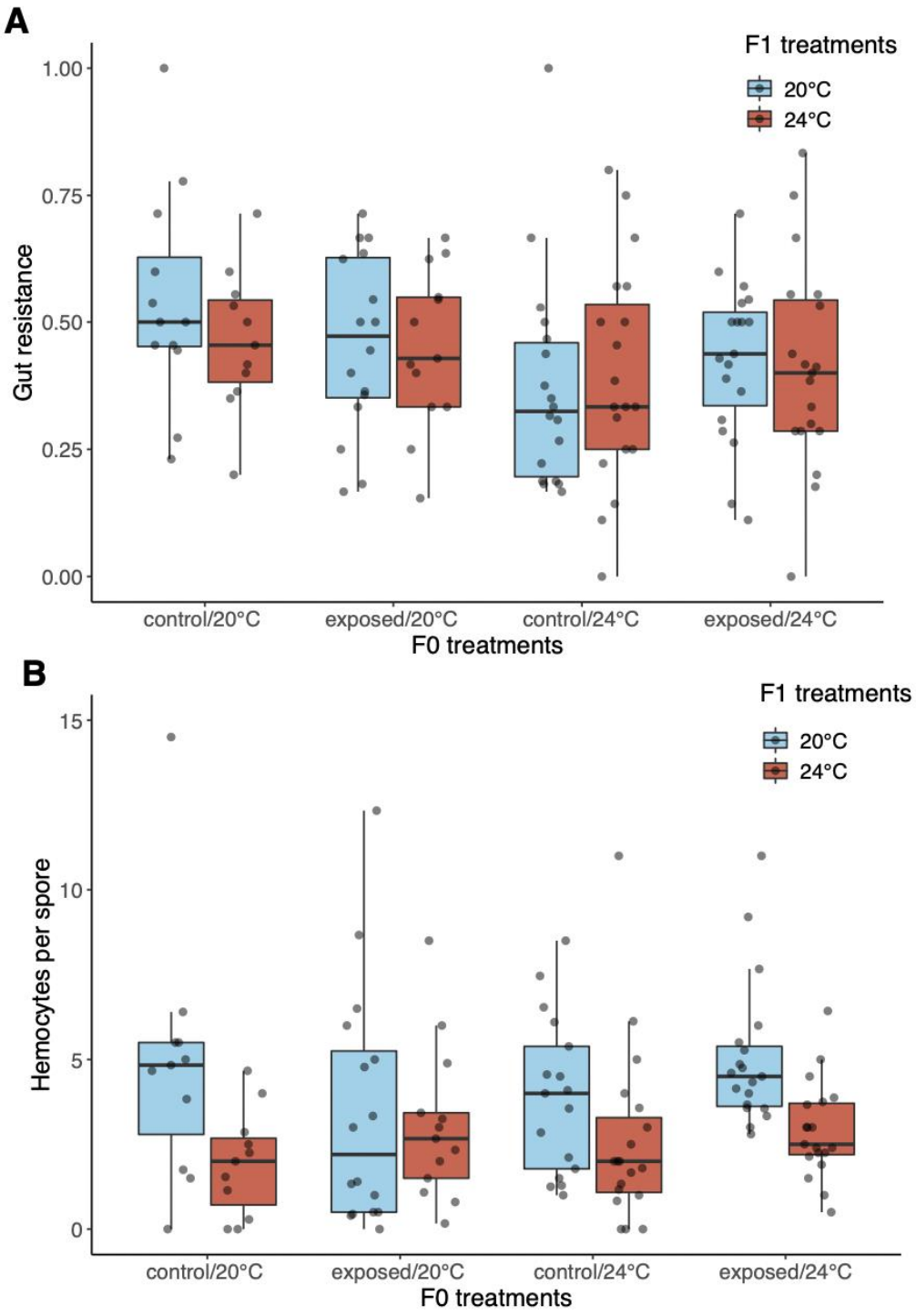

Figure S2. Within- and trans-generational effects of temperature elevation and parasite infection on host defense measured as gut resistance (**A**) and number of hemocytes per spore (**B**). "F0" = parental generation, "F1" = offspring generation. The box plots show median values, the 25th and 75th percentiles, and interquartile ranges.

Table S1. Host defense and life history traits in the offspring generation (F1) explained by the effects of temperature (F0 Temp) and parasite exposure (F0 Para) in the parental generation (F0), and by the effects of temperature (F1 Temp) and parasite exposure (F1 Para) in the offspring generation. Statistically significant p values are highlighted in bold.

Dependent variable	**Explanatory variables**	X^2	**d.f.**	***p* value**
Gut resistance	F0 Temp	3.69	1	0.055
	F1 Temp	0.19	1	0.662
	F0 Para	0.00	1	0.966
	Gut thickness	0.01	1	0.906
Hemocytes per spore	F0 Temp	2.78	1	0.096
	F1 Temp	13.25	1	**<0.001**
	F0 Para	1.30	1	0.254
Age at first reproduction	F0 Temp	6.14	1	**0.013**
	F1 Temp	22.40	1	**<0.001**
	F0 Para	0.003	1	0.960
	F1 Para	0.02	1	0.890
Brood size at first reproduction	F0 Temp	0.03	1	0.855
	F1 Temp	0.09	1	0.767
	F0 Para	0.01	1	0.918
	F1 Para	0.23	1	0.633
Lifespan	F0 Temp	0.78	1	0.377
	F1 Temp	31.21	1	**<0.001**
	F0 Para	0.92	1	0.337
	F1 Para	61.12	1	**<0.001**
	F0 Temp x F1 Temp	5.16	1	**0.023**
	F0 Temp x F0 Para	0.04	1	0.843
	F1 Temp x F0 Para	5.08	1	**0.024**
	F0 Temp x F1 Para	0.21	1	0.644
	F1 Temp x F1 Para	13.51	1	**<0.001**
	F0 Para x F1 Para	2.35	1	0.125
	F0 Temp x F1 Temp x F0 Para	6.88	1	**0.009**
	F0 Temp x F1 Temp x F1 Para	12.63	1	**<0.001**
	F0 Temp x F0 Para x F1 Para	0.81	1	0.370
	F1 Temp x F0 Para x F1 Para	11.10	1	**0.001**
	F0 Temp x F1 Temp x F0 Para x F1 Para	11.36	1	**0.001**

Fecundity	F0 Temp	0.66	1	0.417
	F1 Temp	7.70	1	**0.006**
	F0 Para	0.37	1	0.542
	F1 Para	63.29	1	**<0.001**
	F0 Temp x F1 Temp	0.91	1	0.339
	F0 Temp x F0 Para	0.58	1	0.448
	F1 Temp x F0 Para	2.11	1	0.146
	F0 Temp x F1 Para	0.60	1	0.440
	F1 Temp x F1 Para	10.59	1	**0.001**
	F0 Para x F1 Para	1.82	1	0.177
	F0 Temp x F1 Temp x F0 Para	6.46	1	**0.011**
	F0 Temp x F1 Temp x F1 Para	7.23	1	**0.007**
	F0 Temp x F0 Para x F1 Para	1.12	1	0.291
	F1 Temp x F0 Para x F1 Para	5.97	1	**0.015**
	F0 Temp x F1 Temp x F0 Para x F1 Para	6.65	1	**0.010**

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Table S2. Effects of temperature on the parasite fitness components of infection outcomes in the offspring generation (F1). Statistically significant p values are highlighted in bold.

Dependent variable	**Explanatory variables**	X^2	**d.f.**	***p* value**
Probability of terminal infection	F0 Temperature	1.23	1	0.268
	F1 Temperature	0.01	1	0.907
	F0 Parasite	1.24	1	0.266
Spore yield per host	F0 Temperature	0.02	1	0.897
	F1 Temperature	4.30	1	**0.038**
	F0 Parasite	0.12	1	0.730
	Gut resistance	3.78	1	0.052
	Hemocytes per spore	0.01	1	0.936

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Table S3. Host lifetime fecundity and its relationship with immune responses in the offspring generation (F1) explained by the effects of temperature (F0 Temp) and parasite exposure (F0 Para) in the parental generation (F0), and by the effect of temperature (F1 Temp) in the offspring generation. Statistically significant p values are highlighted in bold.

Dependent variable	**Explanatory variables**	X^2	**d.f.**	***p* value**
Fecundity	F0 Temp	3.03	1	0.082
	F1 Temp	1.80	1	0.179
	F0 Para	0.002	1	0.966
	Gut resistance	1.48	1	0.225
	Hemocytes per spore	5.83	1	**0.016**
	F1 Temp x F0 Temp	11.06	1	**0.001**
	F0 Temp x F0 Para	6.98	1	**0.008**
	F1 Temp x F0 Para	13.30	1	**<0.001**
	F0 Temp x Hemocytes per spore	9.53	1	**0.002**
	F1 Temp x Hemocytes per spore	0.27	1	0.605
	F0 Para x Hemocytes per spore	0.29	1	0.590
	F0 Temp x F0 Para x Hemocytes per spore	6.01	1	**0.014**
	F1 Temp x F0 Para x Hemocytes per spore	10.21	1	**0.001**

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