Transgenerational virulence: Maternal pathogen exposure reduces offspring fitness

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Abstract: There is intense interest in understanding pathogen virulence – that is, the degree of damage caused to a host by a pathogen. However, despite abundant evidence that effects of stressors can carry across generations, studies of pathogen virulence have focused almost exclusively on a single generation. Here, we describe transgenerational virulence, where parental exposure to a pathogen reduces offspring fitness. *Daphnia* host exposure to a microsporidian pathogen significantly reduced lifespan and fecundity of offspring. The transgenerational virulent effects of maternal exposure to the microsporidian pathogen matched or even exceeded the within generation effects of a fungal pathogen that is considered highly virulent. Moreover, they did not result simply from immune activation in the host, as the transgenerational effect was seen for infections by the microsporidian but not for infections by two other pathogens. The transgenerational effects of the microsporidian carried to the granddaughters, with grandmaternal exposure influencing neonate size. A parameterized mathematical model translated these impacts to the population level, finding that transgenerational virulence often reduces host density, infected host density, and infection prevalence, which could have implications for host conservation and spillover risk. Given widespread prior evidence for transgenerational effects of predators, resources, and other factors, we propose that transgenerational virulence may be common.

1 Introduction

By definition, pathogens harm their hosts, and the degree of damage done to the host by the pathogen (known as "virulence") is a major focus of research on host-pathogen interactions. Virulence is traditionally investigated within a generation, measuring the cost of exposure or infection to the exposed/infected individual. However, for many other stressors there is strong evidence of transgenerational effects; for example, maternal predation exposure affects offspring behavior and growth (1, 2, 3, 4) and food restriction can impact offspring size and survival (5, 6, 7, 8). Still, despite the clear evidence of transgenerational effects of

other stressors and the intense interest in understanding pathogen virulence, the potential for transgenerational virulence — that is, reduced fitness of the offspring of exposed or infected hosts — has been overlooked, with potential consequences for individual health, population-level disease dynamics, and spillover risk.

While studies of virulence rarely look across generations, transgenerational effects of infection are well established. In particular, there is substantial evidence that exposure to a pathogen in one generation can alter the immune response of offspring. This transgenerational immune priming allows for hosts that are exposed to a pathogen to produce offspring that are less susceptible to infection (9, 10, 11, 12). Moreover, offspring of exposed hosts can have altered immune profiles (13, 14, 15). Thus, it is clear that the impacts of exposure to pathogens can carry across generations. A few studies have explored the potential for negative effects of infection on offspring survival (16, 17) or population growth (11). However, there has yet to be a clear example of transgenerational virulence due to pathogen infection or exposure. Demonstrating transgenerational virulence would require showing that: first, the impact of infection occurs in offspring that are not themselves infected (which differs from an example like Zika virus, where the offspring becomes infected (18)); second, offspring fitness (e.g., lifespan or reproduction) is reduced; and, third, the impact of maternal infection is not due to the offspring's demise during gestational development (as occurs with SARS-CoV-2 infections where the placenta is damaged (19)).

Here, we demonstrate that exposure of a host to a pathogen reduces fitness of her unexposed and uninfected offspring. We do this using laboratory experiments with *Daphnia* hosts, which are a model system for understanding host-pathogen interactions (20). One advantage of the *Daphnia* system is that individual genotypes can be maintained as asexual clonal lines, with mothers producing daughters clonally. We used three clonal genotypes (referred to as the 'B', 'D', and 'S' genotypes) of *D. dentifera* and a microsporidian, *O. pajunii*, that infects epithelial cells of the host gut. We chose these three genotypes because they are all susceptible to *O. pajunii*, but to different extents. Individuals of each of the three

genotypes were exposed either to *O. pajunii* or to a control solution. After exposure, we collected offspring, which were described as uncompromised offspring (animals with unexposed mothers) or compromised offspring (animals with exposed mothers); importantly, in an additional experiment, we found no evidence of vertical transmission in this system (see Supporting Information). To compare the effects of exposure within and across generations and to also test for transgenerational immune priming, these offspring were then exposed to either *O. pajunii* or a control solution, using a fully factorial design. These individuals from the daughter generation were reared, tracking offspring mortality, reproduction, and infection.

Exposure of mothers to a microsporidian pathogen reduces fitness of unexposed offspring

Exposure of mothers to the pathogen significantly reduced the lifespan of their offspring in two genotypes and marginally in the third (Figure 1A-C; S genotype RMST difference of compromised to uncompromised individuals: -4.141, 95% CI -6.976 to -1.305, p=0.004; D genotype RMST difference of compromised to uncompromised individuals: -4.3, 95% CI -7.477 to -1.123, p=0.008 ; B genotype RMST difference of compromised to uncompromised individuals: -1.844, 95%CI -3.928 to 0.239, p=0.083. See Table S1 for complete statistical results.)

Maternal exposure also impacted offspring production. This effect was particularly strong in the most susceptible genotype (the S genotype), where compromised individuals suffered greatly reduced early life fecundity (i.e., produced fewer neonates in the first 3 clutches; Figure 2A, Table S1, $F(1,36)=16.639$, $p<0.001$) and produced fewer clutches in total (Figure 2B, Table S1, $F(1,36)=7.405$, $p=0.01$). Compromised individuals of the D genotype also produced fewer clutches in total due to maternal exposure, but only when they were exposed to the pathogen (Figure 2C, Table S1, $F(1,36)=4.633$, $p=0.038$). The two genotypes

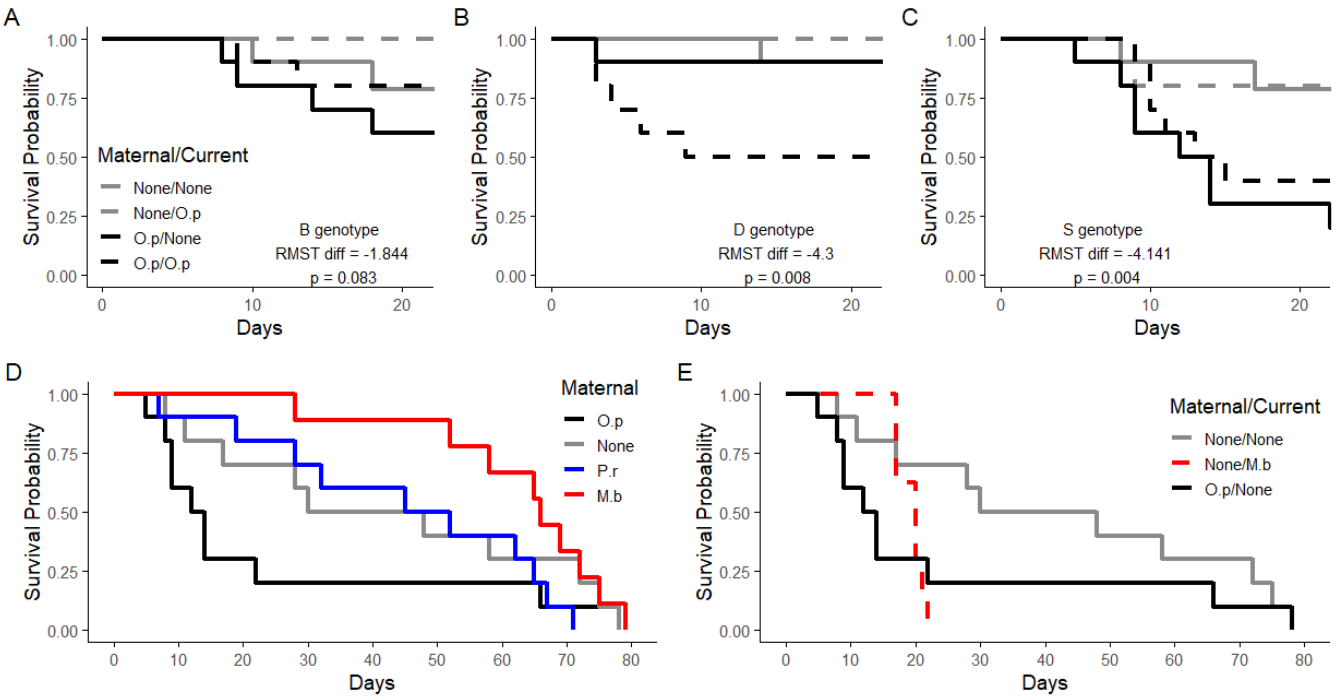

Figure 1: **Daughters of *O. pajunii*-exposed mothers had reduced lifespan.** (A-C) Survivorship curves for the three genotypes, B, D, & S, respectively. Black lines: unexposed offspring of *O. pajunii*-exposed mothers, gray lines: unexposed offspring of mothers that had not been exposed to a parasite; solid lines: no current parasite exposure, dashed lines: current exposure to *O. pajunii*. The RMST difference given is for the impact of maternal exposure on early life survival for each genotype. (D) Unlike offspring of mothers exposed to *O. pajunii*, offspring of mothers exposed to two other common pathogens do not die sooner than offspring of unexposed mothers. None of the offspring in this panel were exposed to a pathogen. *O.p.* indicates *O. pajunii*, *P.r.* is the bacterium *P. ramosa*, and *M.b.* is the fungus *M. bicuspidata*. (E) Uninfected offspring of mothers exposed to *O. pajunii* (black line) experience mortality that is comparable to or even exceeds that experienced by hosts currently infected by *M. bicuspidata* (red dashed line).

experiencing a reduction in reproductive events (S and D) with maternal parasite exposure are also the genotypes with the greatest increase in early life mortality (Figure 1); therefore, it appears that early mortality is driving this reduction in total clutches produced.

Moreover, there was an impact of maternal exposure on the size of neonates produced by daughters for all 3 genotypes (Table S1, Figure 2C). For the B genotype, there was a significant main effect of maternal exposure ($F(1,190)=16.425$, $p<0.001$), and neonates produced by compromised daughters were significantly smaller (Figure 2C), indicating that the effect

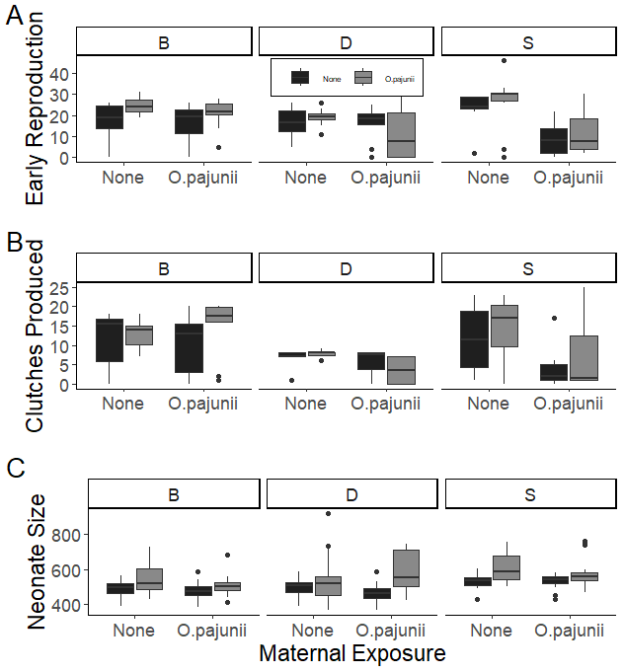

Figure 2: Maternal exposure to *O. pajunii* impacted reproduction in offspring. (A) Early reproduction (total neonates produced in the first three clutches) was strongly reduced by maternal exposure in the S genotype. (B) The total number of clutches produced was reduced by maternal *O. pajunii*-exposure in the D and S genotypes. (C) The size of neonates produced by daughters was impacted by the interaction between maternal and current exposure in the D and S genotypes, and by maternal exposure in the B genotype; this indicates that the impacts of *O. pajunii*-exposure carry over to granddaughters. Black bars represent no current exposure; gray bars indicate current *O. pajunii* exposure.

of exposure carried through to granddaughters. Neonate size is an important predictor of resistance to starvation (21) and future reproductive size and clutch size (22), suggesting potentially reduced reproduction in granddaughters of exposed mothers in this genotype. In both the S and D genotypes, there was a significant interaction between maternal exposure and current exposure on neonate size (S: $F(1,126)=8.454$, $p=0.004$; D: $F(1,128)=11.077$, $p=0.001$). In the D genotype, unexposed, compromised individuals produced smaller offspring. In the S genotype, infected animals produced larger offspring, but only if they were not compromised.

No evidence for transgenerational immune priming

We found no evidence that offspring of exposed mothers were better able to resist infection. We had low power to analyze data on infection prevalence due to high early mortality, which occurred prior to when we assessed infections; however, prevalence was similar in surviving animals in compromised vs. uncompromised treatments for the three genotypes (S genotype: 4/5 compromised vs 8/8 uncompromised infected; D genotype: 1/8 compromised vs 0/8 uncompromised infected; B genotype: 7/8 compromised vs 7/10 uncompromised infected). We also did not find a difference in infection burdens for compromised vs. uncompromised infected (Table S1). Together with the results in the previous section, the results of our primary laboratory experiment indicate costs but not benefits for offspring of exposed mothers.

Transgenerational virulence is pathogen-specific and matches within generation virulence of a highly virulent fungal pathogen

To contextualize our results within this particular system, we conducted two additional comparisons. First, we asked if the transgenerational impact on early mortality was ubiquitous among pathogens. For this, we compared the early mortality due to maternal *O. pajunii* exposure with the early mortality due to maternal exposure to two other common pathogens of *D. dentifera*, *Pasteuria ramosa* (a sterilizing bacterial pathogen) and *Metschnikowia bicuspidata* (a highly lethal fungal pathogen); both of these pathogens are known to stimulate immune responses in exposed hosts (23, 24). We found that only maternal *O. pajunii* exposure increased the risk of early mortality compared to animals with no maternal exposure (Fig 1D). Daughters of both *M. bicuspidata*- and *P. ramosa*-exposed mothers did not differ in their risk of early mortality from daughters of unexposed mothers (*M. bicuspidata*: HR=0.293, 95% CI 0.03-2.822, p=0.288; *P. ramosa*: HR=0.912, 95% CI 0.184-4.519,

p=0.910). Only daughters of *O. pajunii*-exposed mothers suffered increased risk of early mortality (HR=4.654, 95% CI 1.212-17.876, p=0.0251, Figure 1D). Therefore, the transgenerational virulence effect of *O. pajunii* cannot be explained by immune activation caused by exposure to any pathogen. Instead, even within one host species, transgenerational virulence occurs with some pathogens but not others.

We also asked how the strength of early mortality due to transgenerational virulence compared to the mortality imposed by *M. bicuspidata* infection; *M. bicuspidata* is considered highly virulent, with the majority of its virulence stemming from increased mortality (25). We found that our unexposed, compromised animals (that is, unexposed daughters of *O. pajunii*-exposed mothers) suffered a greater risk of early mortality when compared to individuals currently infected with *M. bicuspidata* whose mothers had not been exposed to any pathogens (Figure 1E, RMST difference estimate: -5.163, 95%CI -8.709−1.617, p=0.004). Expanding beyond just early mortality, there were no significant differences in overall time to death between compromised animals and animals infected with *M. bicuspidata* (RMST difference estimate: -10.826, 95% CI -29.563-7.910, p=0.257, Figure 1E). Therefore, the transgenerational virulent impact of maternal exposure to *O. pajunii* on offspring lifespan matches or even exceeds that of the within generation virulence of *M. bicuspidata*.

Transgenerational virulence can reduce total host density, infected host density, and infection prevalence

To explore the implications of transgenerational virulence on host-pathogen dynamics at the host population level, we developed a host-pathogen mathematical model with susceptible and infected uncompromised and compromised individuals and an environmentally transmitted pathogen. We used our model to explore how changes in the mortality, reproductive rates, or infection rates of compromised individuals impact total host density, infected host density, and infection prevalence.

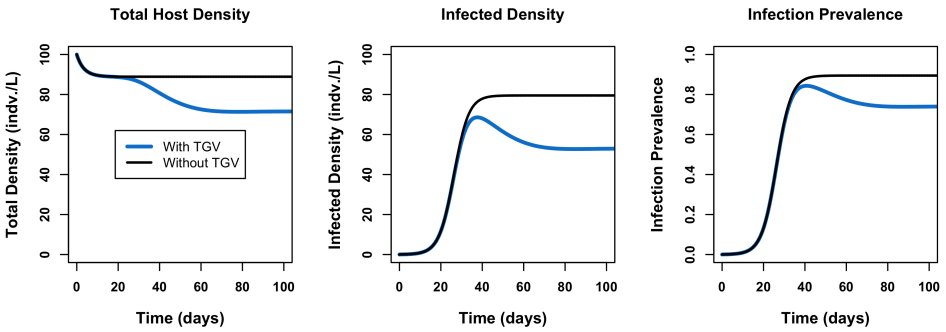

Figure 3: Transgenerational virulence (TGV) is predicted to notably reduce total host density (A), infected host density (B), and infection prevalence (C). Thinner black lines show times series from a model parameterized to the *Daphnia-O. pajunii* system where there is no TGV; thicker blue lines show time series from a model with TGV, where offspring of infected individuals have higher mortality than the offspring of uninfected individuals.

Our model predicts that, if transgenerational virulence manifests as increased mortality or decreased reproduction of offspring of infected mothers, then it will reduce total host density, infected host density, and infection prevalence in most systems (Figures 3, S2-S4, S6-S8); in other words, transgenerational virulent effects on reproduction and survival harm both the host and the pathogen at the population level. If transgenerational virulence manifests as increased infection rates by offspring of infected mothers, then it will harm the host population but benefit the pathogen in most systems: increased infection rates for compromised individuals decreased total host density, while infected host density and infection prevalence both increased (Figures S2-S4, S6-S8). When parameterized for the *Daphnia-O. pajunii* system, the model predicted that the observed increase in mortality of susceptible and infected compromised individuals due to transgenerational virulence reduced total host density by 30.3% (Fig. 3A), infected host density by 45.4% (Fig. 3B), and infection prevalence by 21.7% (Fig. 3C). Additional details about the model, the above predictions, and exceptions are provided in the Supporting Information.

Discussion

While evolutionary ecologists have long recognized that the effects of stressors can span generations, the potential for pathogen virulence to span generations has been overlooked. This is a concern because it has potential implications for individual health, population size and health, and the risk of disease spillover to community members. Here, we show that exposure to a microsporidian pathogen impacted the fitness of the unexposed daughters of exposed mothers. Notably, the effect of maternal exposure to the microsporidian on offspring lifespan was similar to the within-host virulence of a fungal pathogen that is considered highly virulent (due primarily to its impact on host lifespan). The reproduction of the daughters of exposed mothers was also affected; the most susceptible genotype showed particularly strong reductions in the number of offspring produced by the daughters of exposed mothers. Our parameterized mathematical model reveals the potential population- and community-level impacts of transgenerational virulence: increased mortality or reduced fecundity of the offspring of exposed mothers can reduce total host density, infected host density, and infection prevalence.

A major concern at the intersection of disease and community ecology is the potential for pathogen spillover (i.e., transmission of pathogens to uninfected host populations or species (26)). Accurate assessment of spillover risk is critically important, as more than 60% of emerging infectious disease stems from spillover (27). Host density is a significant factor in spillover risk (28), and the decreases in total host density predicted by our model may decrease spillover. Transgenerational virulence also reduced infected host density, which should further reduce spillover risk in systems with density-dependent transmission (29). Therefore, transgenerational virulence-related density changes could alter outbreak prediction and management success (30). Moreover, in cases where a host population is of conservation concern, transgenerational virulence might have mixed impacts at the population level: if the impacted host is the one of conservation concern, transgenerational virulence should exacerbate the impacts of infection, but if infections from the impacted host spillover to a

host of conservation concern, transgenerational virulence should reduce spillover risk.

Studies on transgenerational effects of other stressors point to potential mechanisms that might underlie our empirical findings. First, molecular mechanisms, such as epigenetic inheritance, often influence transgenerational shifts in response to stressors, as evidenced in a wide variety of taxa; for example, environmental stress-induced epigenetic changes negatively impact offspring fitness in Polpay sheep (due to diet stress, (31)) and *C. elegans* (due to toxicant exposure, (32)). Second, organisms can shift energetics in response to stressors; for example, altering reproduction under conditions of environmental stress (or parasitism) is a well characterized strategy to maximize fitness. While this is commonly seen as changes in the number of offspring (33, 34), it can also manifest as alterations of nutrients maternally partitioned to offspring (as in *Daphnia*, with reduced egg fat content after predator signals (35, 36)). In our system, pathogen exposure does not lead to reduced maternal investment via embryo production or yolk size (Figure S1), though this does not rule out changes in specific chemical composition of compromised offspring. Indeed, there is a signal in our data that the embryos of compromised mothers are different: embryos that were removed from the brood chamber were much less likely to complete development if they came from exposed mothers (Figure S1A). Additionally, these two mechanisms (i.e., molecular and energetic shifts) may combine: in fruit flies, high sugar maternal diets altered body composition of offspring and modified metabolic gene expression, with fitness impacts carrying throughout the offspring lifespan (37). Temporarily reducing reproductive investment after pathogen exposure may provide more resources for immune response, a key strategy for combating pathogens like *O. pajunii* because of its relatively low (within generation) virulence that does not pose an immediate mortality risk (38). We hypothesize that pathogen exposure caused mothers to reduce investment in embryos, yielding offspring with reduced early survival. This would result in the appearance of similar reproductive success for the parent generation, with costs of exposure manifesting as transgenerational virulence in the offspring generation. In our system, the most notable impact was on early mortality of offspring of exposed

mothers, consistent with the paradigm that any reduction in fitness due to reduced maternal investment would manifest early in life (39).

While there was a clear overall pattern of transgenerational virulence in our study, the manifestation differed somewhat among genotypes. The impact on daughter lifespan and reproduction was particularly strong for one genotype (S), the effect on granddaughter size was particularly strong for another genotype (B), and the third genotype (D) suffered intermediate impacts on lifespan, reproduction, and granddaughter size. This difference among genotypes in transgenerational impacts resembles the variation shown by *Drosophila* genotypes in transgenerational responses to environmental stressors (40). Likewise, the genotypic variation in persistence of response (through second or third generation) is similar to variation found in *Arabidopsis* (41), with impacts of the environment varying by genotype and even varying within genotype by generation. These differences have the potential to scale up to host population-level differences in the ability to compensate for transgenerational virulence-driven increases in mortality (42), with potential implications for host population genetic composition and infection prevalence – providing further evidence of the importance of considering host life history in disease ecology (43).

Our experiment focused on transgenerational impacts of pathogen exposure, rather than on infection. Here, offspring of exposed mothers suffered reduced fitness even though there were no obvious signs of infection in the mothers at the time the offspring were produced. This is consistent with earlier work that found reduced fitness in bumblebees within the same generation, where individuals who were exposed to a sham infection that stimulated the immune system had greatly reduced survival (44). Thus, our work adds to evidence that virulence costs may start accumulating upon exposure, and extends this to show those impacts can span generations. In showing an effect of exposure that spans generations, it is similar to work on other natural enemies, including work that shows exposure to parasitoid wasps can cause *Drosophila* to alter reproductive behaviors, with consequences for offspring fitness (45).

Here, we demonstrated that exposure to a microsporidian pathogen has detrimental effects on the fitness of offspring of the exposed hosts. This transgenerational virulence manifested as decreased survival and reduced fecundity of offspring of exposed mothers. These individual-level transgenerational impacts of pathogen exposure were similar to the within generation impacts of a different, highly virulent pathogen. These effects were pathogen-specific: we saw reduced fitness of offspring of mothers that were exposed to a microsporidian pathogen, but not for offspring of mothers exposed to two other pathogens. The transgenerational virulence we measured for the microsporidian should have impacts at the population-level: our model reveals that transgenerational virulence can notably decrease host density, infected host density, and infection prevalence. Given the widespread prior evidence for transgenerational effects of stressors, we propose that transgenerational virulence is likely to occur in other host-pathogen systems, with consequences for both hosts and pathogens.

Data Archival

Data and code are available on GitHub (https://github.com/kmmcintire/TGV).

Primary laboratory experiment methods

Daphnia dentifera is a zooplankton species commonly found in North American lakes (46) and, along with other species of *Daphnia*, is a model system for studying host-pathogen interactions (20, 47). We used three clonal genotypes, including the 'Standard' genotype (hereafter 'S' genotype), originally collected from a lake in Barry County, MI; this clone is highly susceptible to our focal pathogen and has been studied extensively. In addition to the 'S' genotype, we used two additional clonal genotypes 'BD08-46' and 'DW 22-58' (both originally collected from lakes in Greene-Sullivan State Forest, Indiana, hereafter labeled 'B' and 'D', respectively). These genotypes were tested as they are also known to have some level of susceptibility to our pathogen, though much lower than the S genotype (38). This allowed us to explore the generality of the patterns that we observed. The focal pathogen,

Ordospora pajunii, is a microsporidian formerly known as 'MicG' that is common in lake populations of *D. dentifera* in the Midwestern United States (48). *O. pajunii* infects cells in the host gut epithelium. It produces transmission stages that are released into the host's digestive tract and, subsequently, into the water column along with host feces (M.A. Duffy et al., unpublished data). *Daphnia* encounter and ingest these transmission stages while grazing for phytoplankton, after which the pathogen may infect the host via the gut wall.

We acclimated the three clonal genotypes of *D. dentifera* for three generations in common conditions to homogenize host environmental history and minimize unintended variation in maternal effects (7). The Supporting Information gives more specific methods for this experiment. Briefly, mothers of each genotype were raised under standardized conditions and then half of the mothers of each genotype were exposed to a spore slurry created from *O. pajunii*-infected individuals; control animals were exposed to a similar slurry created from uninfected individuals. Daughter generation individuals were collected as 24 hr old neonates from the mothers' first clutch. Each daughter was assigned to one of two exposure treatments: *O. pajunii* or placebo. This was done in a factorial design, such that daughters from each maternal exposure treatment were exposed to either daughter exposure treatment (for a summary of experiments, see Supporting Information).

For the daughter generation, we recorded time to first reproduction, number of neonates produced in each of the first 3 clutches, total number of clutches produced over the entire lifespan (as a measure of total reproduction), body length at time of first reproduction, infection status (yes/no and burden, on Day 14 and every 5 days thereafter), and date of death. We recorded neonate abundance for clutches 1-3, as these clutches were considered most ecologically relevant (based on mortality rates in the field, which are often 0.1-0.2 day^{-1}; (49)) and have the strongest impact on host population rate of change. We also measured the length of neonates in the first clutch, which we consider a measure of maternal reproductive investment (along with clutch size) and offspring fitness, as neonate size positively correlates to future size at reproduction and time to reproduction and larger neonates are more resistant

to starvation (21, 50). For both body length measurements (on daughters at time of first reproduction and of their first clutch), size was measured as the length from the center of the eye to the base of the tail spine. All of the animals that made clutches had done so by day D20. Therefore, because we were particularly interested in early life fitness (lifespan through the first three clutches), we truncated lifespan (that is, day of death) data at Day D20.

Analysis of primary laboratory experiment

To explore the potential for transgenerational virulence, we tested for differences in host fitness between maternal pathogen exposure treatments for unexposed daughters. This was done to address whether maternal pathogen exposure negatively impacted daughter fitness and, if so, to determine how host fitness components were impacted. We analyzed body size at first reproduction, clutch 1 neonate size, clutch 1 neonate abundance, total clutch 1-3 reproduction, and total number of clutches produced using non-parametric aligned rank transformation ANOVA with maternal exposure, current exposure, and their interaction as fixed effects (ARTtools, (51)). We analyzed time to first reproduction and risk of early mortality with a time to event analysis, with maternal exposure, current exposure and their interaction as fixed effects (survival analysis, Cox Proportional Hazard). This analysis yielded infinite interaction values for genotypes B and D in the analysis for risk of early mortality; therefore, we conducted and report restricted mean survival time analysis (R package survRM2, (52)). As the RMST result did not qualitatively differ from the hazard model for the S genotype, we report this result for all three genotypes.

To explore the potential for transgenerational immune priming, we tested for differences in daughter susceptibility between maternal pathogen exposure treatments for our *O. pajunii*-exposed daughters. We did not formally analyze data on infection prevalence because few animals lived long enough for us to accurately assess infection. We used a Kruskal-Wallis test to test for an effect of maternal exposure on total infectious burden. We analyzed time

to infection using a time to event analysis, with maternal exposure as a fixed effect (survival analysis, Cox Proportional Hazard).

Analyses for all clones were conducted independently, using the same methods. The exception to this is the infectious burden analysis for genotype D which was not conducted due to lack of productive infections. All data were analyzed in R version 4.2.3. Statistical results from the laboratory experiments can be found in Table S1.

Additional maternal pathogen exposure methods

We carried out additional analyses to investigate the potential for other pathogens to cause TGV in this host, and also to compare the transgenerational virulence of *O. pajunii* with the within generation virulence of another pathogen that is considered highly virulent. For the former, we used two other common, well studied pathogens of *D. dentifera*, *Pasteuria ramosa* and *Metschnikowia bicuspidata*, both of which are known to trigger strong immune responses in hosts (23, 24). For the latter, which used *M. bicuspidata*, which is generally considered highly virulent due to heavily increased early mortality in infected hosts (25). This experiment was conducted on S genotype mothers because they are susceptible to all three of these pathogens. Additional experimental methods are given in the Supporting Information.

First, we were interested in comparing the transgenerational impact on mortality of exposure to *O. pajunii* with that of exposure to *P. ramosa* and *M. bicuspidata*. To do so, we analyzed risk of early mortality using a time to event analysis with maternal exposure as a fixed effect; we elongated the truncation time for this analysis (survival analysis, Cox Proportional Hazard) to Day 28 as that was the day of first death for one of the treatment groups (offspring of *M. bicuspidata*-exposed mothers). Second, we wished to compare the transgenerational virulence of *O. pajunii* to the within generation virulence of *M. bicuspidata*. To do this, we compared risk of early mortality (truncation time day 20) and overall time to death for animals infected with *M. bicuspidata* and uninfected animals compromised by

maternal *O. pajunii* exposure. We used the restricted mean survival time method (R package survRM), as data did not fit a proportional hazards model.

Mathematical modeling of transgenerational virulence

To explore the population-level effects of transgenerational virulence, we analyzed an SIR-type environmental transmission model that describes the dynamics of the densities of susceptible (S) and infected (I) individuals who are the offspring of susceptible individuals, susceptible (C) and infected (D) individuals who are the offspring of infected individuals (hereafter, compromised and decimated individuals, respectively), and infectious propagules in the environment (P). As in the *Daphnia-O. pajunii* system, infection occurs when susceptible or compromised individuals encounter infectious propagules that were shed previously into the environment by infected or decimated individuals.

The model equations are

$$\frac{dS}{dt} = \overbrace{Sf_S(N) + Cf_C(N)}^{\text{reproduction}} - \overbrace{\beta_S SP}^{\text{infection}} - \overbrace{m_S S}^{\text{mortality}} \tag{1a}$$

$$\frac{dI}{dt} = \overbrace{\beta_S SP}^{\text{infection}} - \overbrace{m_I I}^{\text{mortality}} \tag{1b}$$

$$\frac{dC}{dt} = \overbrace{If_I(N) + Df_D(N)}^{\text{reproduction}} - \overbrace{\beta_C CP}^{\text{infection}} - \overbrace{m_C C}^{\text{mortality}} \tag{1c}$$

$$\frac{dD}{dt} = \overbrace{\beta_C CP}^{\text{infection}} - \overbrace{m_D D}^{\text{mortality}} \tag{1d}$$

$$\frac{dP}{dt} = \overbrace{\chi_I I + \chi_D D}^{\text{shedding}} - \overbrace{uPN}^{\text{uptake}} - \overbrace{\delta P}^{\text{degradation}} \tag{1e}$$

where $N = S + C + I + D$ is the total host density. In equation (1a), susceptible density increases due to reproduction by susceptible (Sf_S) and compromised (Cf_C) individuals and decreases due to infection ($\beta_S SP$) and non-disease mortality ($m_S S$). In equation (1b), infected density increases when susceptible individuals become infected ($\beta_S SP$) and decreases

due to mortality from disease and non-disease sources ($m_I I$). In equation (1c), compromised density increases due to reproduction by infected individuals ($I f_I$) and decimated individuals ($D f_D$) and decreases due to infection ($\beta_C CP$) and mortality due to non-disease sources ($m_C C$). In equation (1d), decimated density increases when compromised individuals become infected ($\beta_C CP$) and decreases due to mortality from disease and non-disease sources ($m_D D$). In equation (1e), infectious propagule density increases due to shedding by infected and decimated individuals ($\chi_I I + \chi_D D$) and decreases due to uptake by all individuals (uPN) and degradation (δP). Importantly, to account for the transgenerational effects of infection, when compared to susceptible individuals, compromised individuals have (i) equal or higher mortality rates ($m_C \geq m_S$), (ii) equal or lower reproductive output ($f_C \leq f_S$; see below), and (iii) higher, equal, or lower transmission rates ($\beta_C > \beta_S$, $\beta_C = \beta_S$, or $\beta_C < \beta_S$, respectively).

The simplifying assumptions built into our model include infected and decimated individuals shed identical infectious propagules, susceptible and compromised offspring have identical offspring (i.e., there are no grandmaternal effects of infection), and all individuals have equal uptake rates (u). In addition, we assume per capita reproduction rates only depend on total host density and are decreasing functions of total host density, e.g., $f_S(N) = r_S f(N)$ where $f'(N) < 0$. Moreover, infection causes infected and decimated individuals to have lower reproduction rates than susceptible and compromised individuals, respectively ($f_I \leq f_S$ and $f_D \leq f_C$).

In the Supporting Information, we explore the population-level effects of transgenerational virulence (i.e., increased mortality, m_C, lower reproductive output, f_C, and unequal transmission rates, β_C, for compromised individuals) by computing explicit formulas for the local sensitivities of total host density (N), total infected density ($I + D$), and infection prevalence ($[I + D]/N$) at equilibrium to the model parameters. Because the high dimensionality of model (1) limits the tractability of the sensitivity formulas, we also analyze a reduced model that assumes infected and decimated individuals are identical. The reduced model facilitates interpretation by reducing the complexity of the sensitivity formulas; the

predictions from the two models qualitatively agree.

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