ORIGINAL ARTICLE

Contrasting parasite-mediated reductions in fitness within versus between patches of a nematode host

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Host and parasites interact across spatial scales, but parasite-mediated fitness effects are typically measured only at local scales. Recent work suggests that parasites can reduce host fitness during dispersal between patches, highlighting the potential for both within- and between-patch effects to contribute to the net fitness consequences of parasitism. Building on this work, we measured the contribution of the dispersal phase to parasite-mediated reductions in host fitness. We used the nematode *Caenorhabditis elegans* and its natural microsporidian parasite *Nematocida parisii* to quantify the fitness consequences of parasitism at the individual, population, and metapopulation level. *Nematocida parisii* reduced individual fecundity and population growth but had its greatest fitness impact at the dispersal stage: parasitism reduced the fitness of dispersing larvae by 62%–100%. These results indicate that the cost of parasitism in this system is greatly underestimated if the metapopulation level is not taken into account. We also found that the effects of *N. parisii* vary with host genotype, and the relative advantage of the most resistant genotype increases with inclusion of the dispersal stage. Taken together, our findings demonstrate that host-parasite interactions at the dispersal stage can magnify selection for parasite resistance.

KEY WORDS: *Caenorhabditis elegans*, disease, dispersal, host-parasite interactions, metapopulation dynamics, microsporidia, migratory culling, *Nematocida parisii*.

Host-parasite interactions unfold across a network of patches connected by dispersal (i.e., a metapopulation). Incorporating metapopulation structure has advanced our understanding of the dynamics (Dwyer 1991; Grosholz 1993; Antonovics 2004; Lopez et al. 2005) and evolution (Boots and Sasaki 1999; Morgan et al. 2005; Jousimo et al. 2014) of host-parasite interactions. For simplicity, most metapopulation models do not account for individual host movement and colonization. Instead, these models assume that dispersers mirror their populations of origin and are not differentially affected by parasites while in transit (Daversa et al. 2017). This approach assumes that the fitness cost of infection can be estimated from within-patch metrics alone. In this study, we evaluate this assumption by quantifying parasite-mediated reductions in host fitness components within and between patches and assessing their relative contribution to the overall fitness effects of parasitism.

Some empirical data suggest that parasites impose distinct costs at the host's dispersal stage (Fellous et al. 2011; Terui et al. 2017; Baines et al. 2020). Infection can reduce a host's probability of dispersing successfully or prevent dispersal altogether (Fellous et al. 2011; Debeffe et al. 2014; Risely et al. 2018; Baines et al. 2020). For example, monarch butterflies infected with the protozoan parasite *Ophryocystis elektroscirrha* have reduced flight performance (Bradley and Altizer 2005) and wing strength (Davis and de Roode 2018), likely increasing mortality during long-distance migration.

Parasite-mediated dispersal costs may impact the evolutionary trajectory of the host-parasite interaction. If dispersal costs are large and dispersal is frequent, the dispersal phase could contribute substantially to the total fitness cost of infection and impose additional selection for host defenses at the metapopulation level. Estimated dispersal costs of parasitism have generally been

low (Risely et al. 2018), but most studies have focused on seasonal long-distance migration (Dingle and Drake 2007) in taxa that are difficult to track across scales. Understanding the evolutionary relevance of parasite-mediated dispersal costs necessitates a study system that can be monitored both within and between patches so as to draw cross-scale comparisons.

In this study, we investigated the microsporidian parasite *Nematocida parisii* as a source of selection on the model nematode *Caenorhabditis elegans* at the level of the patch and the metapopulation. First, we quantified parasite-mediated reductions in survival, fecundity, and population growth. Second, we measured parasite-mediated effects on the *C. elegans* dispersal stage, known as dauer. We assayed multiple genotypes to determine if genetic variation in parasite resistance varies between scales. In addition to within-patch fitness consequences, we found that parasite exposure strongly reduced *C. elegans* dispersal success, arguing that metapopulation structure can magnify the costs of parasitism beyond that estimated at the patch level alone.

Materials and Methods
NATURAL HISTORY
Caenorhabditis elegans inhabits ephemeral resource patches, dispersing between them via a specialized dispersal life stage called dauer (Cassada and Russell 1975). When conditions are favorable, young larvae develop normally and become reproductive adults within a few days. When the environment becomes crowded, resource limited, or otherwise stressful, young larvae instead enter the dauer stage, which is characterized by stress resistance and a longer life span than under normal development (Cassada and Russell 1975; Klass and Hirsch 1976). These dauers disperse to new patches, such as rotting fruits or stems, via invertebrate vectors and "recover," resuming normal development into reproductive adults (Félix and Duveau 2012). This dispersal mechanism suggests that *C. elegans* is a "passive" disperser with the primary energetic cost likely incurred in development into and out of the dauer stage, rather than during transit itself (Bonte et al. 2012). Most *C. elegans* individuals are self-compatible hermaphrodites (Félix and Braendle 2010). Therefore, a small number of dauers (3–10) can colonize a new patch (Richaud et al. 2018). Although it is common to find patches containing only one genotype, coexistence of multiple genotypes can occur within and among nearby patches (Richaud et al. 2018). Thus, competition between *C. elegans* genotypes plays out at two levels, the patch and the metapopulation.

The microsporidia *Nematocida parisii*, a natural parasite of *C. elegans*, likely contributes to the outcome of nematode competition within patches. The parasite is transmitted horizontally through a fecal-oral route in which infected hosts shed parasite spores into the environment that are then consumed by uninfected hosts (Troemel et al. 2008). *Nematocida parisii* is found throughout the natural range of *C. elegans* (Zhang et al. 2016). Infection can reduce fecundity and survival (Troemel et al. 2008; Balla et al. 2015) and reverse the outcome of competition between two genotypes under lab conditions (Richaud et al. 2018). Together, these studies suggest that *N. parisii* is an ecologically relevant selective force for *C. elegans*.

STRAINS AND CULTURING
We used seven strains of *C. elegans*. Five strains were collected in France and identified as having active *N. parisii* infections when isolated: JU1249, JU1762, JU2132, JU2287, and JU2816 (Zhang et al. 2016). We established parasite-free lines of these strains for maintenance in the lab. We also included the standard lab strain N2 and the well-studied wild strain CB4856 that have low and high levels of resistance to *Nematocida* infection, respectively (Balla et al. 2015, 2019). For experiments requiring monitoring of individual hosts, we used four of these strains—N2, CB4856, JU1762, and JU2132—that were phenotypically distinct based upon prior work (Balla et al. 2015) and preliminary estimates of population growth rates. Strains were thawed from −80°C frozen stocks and maintained at 20°C on Nematode Growth Medium (NGM Lite) plates seeded with *Escherichia coli* OP50 for approximately four generations prior to each experiment. All experiments were conducted at 20°C on *E. coli* OP50-seeded NGM plates.

We used the ERTm1 strain of *N. parisii* that was isolated from *C. elegans* strain ERT002 (originally CPA24) in Franconville, France (Troemel et al. 2008; Balla et al. 2015). To generate stocks of *N. parisii*, we infected large populations of *C. elegans* genotype ERT54 with *N. parisii* and collected infected hosts and spores by washing plates with M9 buffer. ERT54 contains a construct that induces GFP expression upon *N. parisii* infection, facilitating rapid validation of infection in our stock cultures (Bakowski et al. 2014). We serially diluted the host/spore suspensions and plated them on LB and NGM plates. If no contaminants grew after 1 week at 20°C, the suspension was deemed clean and pooled with other clean suspensions. We ground clean suspensions with silicon carbide beads to extract spores, and filtered them at 5 μm to remove debris. We checked again for contaminants as above, then froze clean stocks at −80°C. The process was repeated with parasite-free host populations to acquire control lysate. We quantified the spore concentration of stocks by staining spores with Calcofluor-White and counting them on a Cell-VU® slide.

With the exception of Dispersal Experiment 1 (see below), we used spores from a single stock throughout this project. This stock had an estimated concentration of 374,994 spores/μL. For assay of host survival, fecundity, and population growth, we

exposed groups of 500 L1 larval hosts to 50 µL of spore solution on 60-mm plates (details below). This translates to 1.9×10^7 spores per exposure plate, a number roughly comparable to prior studies (Szumowski et al. 2014; Balla et al. 2015) that results in the majority of hosts (>60%) having visibly proliferating infections at 24 h (unpublished data). For the dispersal experiments, we used a range of spore solution volumes and exposure timings to generate variation in exposure dose (details below). In general, we opted for lower volumes of spore solution in these dispersal experiments because the design allowed for multiple rounds of parasite proliferation that amplified exposure dose.

MEASURING HOST SURVIVAL

To quantify the consequences of parasite exposure on host survival, we monitored survival of the host genotypes N2, CB4856, JU1762, and JU2132 in the presence and absence of *N. parisii*. We collected eggs of each host genotype using a standard bleach wash. Twenty-four hours later, the hatched L1 larvae were washed from plates into M9 buffer and quantified. For each genotype, we mixed 500 L1s with either 50 µL *N. parisii* solution (exposed treatment) or 50 µL control lysate (control treatment) and deposited the mixture onto 60-mm plates seeded with OP50. After 24 hours, we transferred hosts to assay plates: for each combination of host genotype and treatment, we moved 25 hosts onto twenty-five 35-mm plates such that each plate contained one individual. We checked each host daily for survival and transferred it to a new plate every other day for the first 8 days to separate it from its offspring. We censored any individuals who went missing or accidentally suffered damage unrelated to the test conditions ($n = 12$ of 200). Replication structure, sample sizes, and censored individuals for this and subsequent assays can be found in Table S1. For this assay and all following assays, experimenters were blind to experimental conditions while collecting data. We fit a Cox proportional hazards model to the data using the Surv() and coxph() functions in the R package "survival" (Therneau and Grambsch 2000; Therneau 2021). We included host genotype, treatment, and their interaction as predictors. For this and subsequent analyses, we compared full models to models lacking predictors of interest using AIC and Akaike weights. We identified the best model(s) as those with ΔAIC below 2 and weights near 1. We conducted all statistical analyses in R (version 3.6.0; R Core Team 2017).

MEASURING HOST FECUNDITY

To quantify the consequences of parasite exposure on host reproduction, we measured offspring production of N2, CB4856, JU1762, and JU2132 in the presence and absence of *N. parisii*. As above, we established control and exposed treatments, terminated exposure after 24 h, and moved 25 hosts per host genotype per treatment onto 35-mm plates such that each plate contained

one individual. We let each host lay eggs for 24 h before transferring it to a new plate. We repeated these transfers for 7 days, at which point hosts had finished reproducing. Plates with eggs were incubated at 20°C for 24 h, then scored for viable progeny using an aspirator to avoid double counting. Hosts were censored if they could not be found on their plate or were male ($n = 6$ of 200) (Table S1). To determine if parasites reduced lifetime fecundity, we summed an individual's daily fecundity prior to analysis. Our initial Poisson regression was overdispersed, so we fit a negative binomial regression with host genotype, treatment, and their interaction as predictors of variation in individual hosts' lifetime fecundity. To determine if parasite exposure delayed reproduction, we fit a Poisson regression with host genotype, treatment, day of reproduction, and two-way interactions of treatment with host genotype and day as predictors of variation in individual hosts' daily fecundity. We included random effects for individual hosts as well as for each observation of daily fecundity to correct for overdispersion.

MEASURING POPULATION GROWTH

We then quantified population growth in the presence and absence of parasites to estimate the combined effects of variation in host reproduction, timing of reproduction, and survival. We included seven host genotypes (N2, CB4856, JU1249, JU1762, JU2132, JU2287, and JU2816) and established control and exposed populations as above. After 24 h of exposure, we established each population by moving a single host onto a 100-mm plate and allowing it to reproduce for 6 days, capturing two to three host generations (Feistel et al. 2019). The resulting population was collected in M9, washed, and brought to a volume of 14.5 mL. We then counted the number of hosts in six 20-µL aliquots per population. We replicated each combination of host genotype and treatment 20 times, except for the genotype JU2287, which was only replicated 10 times per treatment. We assayed population growth in three separate blocks. We censored replicate populations if the founding host was male or experienced nonfocal damage ($n = 3$ of 260) (Table S1). We fit a Poisson regression with block, host genotype, treatment, and an interaction between host genotype and treatment as predictors of variation in host number per 20-µL aliquot. We included random effects for replicate population as well as each observation (aliquot) to correct for overdispersion.

MEASURING DISPERSAL

We conducted a series of experiments to determine if parasites reduce the dispersal success of hosts (Fig. S1). Replication structure, sample sizes, censored individuals, and males for these experiments can be found in Table S2. Our general approach was to establish host populations with and without parasites, extract dauer individuals several days later when the

population became crowded, transfer dauers to individual plates, and monitor them for development and reproduction. We quantified dispersal success as the fraction of dauer hosts that resumed normal development and reproduced after dispersal, indicative of their potential to successfully colonize new patches. With this approach, we cannot detect effects of parasites on the transit stage of dispersal, during which a dauer larva must find, attach to, and survive on an invertebrate carrier. We focus on the recovery stage of dispersal because it is experimentally tractable and is a critical step for passive dispersers like *C. elegans*. We anticipate that incorporating steps in the transit stage would magnify the negative effect of parasites on dispersal success.

In Experiment 1, we tested for effects on dispersal following exposure of the N2 genotype to relatively large parasite doses. We obtained L1 hosts as above. For this initial experiment, we used a different spore stock with an estimated concentration of 44,254 spores/µL. All other dispersal experiments used the same spore stock as the fitness assays described above. We established three replicate exposed populations by combining 500 L1s with 100 µL of spore solution on 60-mm plates seeded with *E. coli* OP50. We varied the concentration of the spore solution (100%, 10%, and 1% for a range of 4×10^6 to 4×10^4 spores/plate) across these three replicates by diluting with control lysate; however, over the course of 10 days, multiple rounds of parasite proliferation likely generated large exposure doses for dauer larvae in all three exposed populations (Fig. S1). Thus, for the purposes of the initial experiment, we treated these three populations as replicates of a high dose exposure. Consistent with this, we observed very little variation in dispersal success between exposed replicates. We also established two replicate control populations by combining 500 L1s with 100 µL control lysate. Ten days later, we identified dauers by eye, moved them to new plates such that each plate contained one individual, and monitored them daily for developmental stage and reproduction. We monitored 20 dauers per replicate population, giving 40 dauers in total for the control treatment and 60 for the exposed treatment. Dauers were censored if they could not be found on their plate ($n = 3$ of 100) (Table S2).

In Experiment 2, we manipulated timing of parasite exposure to generate broader variation in parasite dose with the goal of determining if the effects of parasitism on dispersal persisted at lower doses. We obtained eggs as before and allowed these to develop to the fourth larval (L4) stage. Host populations were initiated with 10 L4s and then inoculated with spores (10 µL of spore solution diluted with 70 µL M9 buffer) or control lysate (10 µL control lysate diluted with 70 µL M9 buffer) on 60-mm plates that day, 3 days later, or 6 days later. By varying the timing of inoculation, we created variation in parasite dose experienced by hosts later in the growth of the host population, when

the vast majority of dauers develop. For example, inoculation on the day we established host populations allowed for multiple rounds of parasite amplification and transmission prior to the end of the experiment on day 10, ensuring a large exposure dose for most hosts developing as dauers; inoculation 6 days after population establishment allowed for only a single round of parasite transmission and thus a relatively small exposure dose. We replicated each combination of timing and treatment three times. Ten days after we initiated host populations, we collected hosts in M9 buffer and treated them with 1% sodium dodecyl sulfate (SDS) for 15 min. SDS kills non-dauers, providing a rapid and reliable method for isolating dauers (Cassada and Russell 1975). Infected dauers maintain their infections after SDS isolation (unpublished data). We washed dauers with M9 three times to remove excess SDS, plated them, and then moved live dauers onto 35-mm plates such that each plate contained one individual. We monitored dauers daily to assess developmental stage and reproduction. We followed 20 dauers per replicate population, giving 60 total for each combination of timing and treatment. Dauers were censored if they could not be found on their plate or were male ($n = 3$ of 360) (Table S2).

In Experiment 3, we included multiple genotypes to determine if the effects of parasitism on N2 dispersal were general. We performed the experiment as described in Experiment 2 with a few modifications: we used four host genotypes (N2, CB4856, JU1762, and JU2132), we gave host populations spore solution or control lysate on the same day we initiated host populations, and we isolated dauers 7 days later to replicate a medium exposure dose. Each combination of host genotype and treatment was replicated three times and we assayed 20 dauers per replicate population, giving 60 dauers total per combination of host genotype and treatment. Dauers were censored if they could not be found on their plate ($n = 8$ of 480) (Table S2).

We scored dispersal as successful when an individual produced offspring on the new plate, so only hermaphrodites could be scored easily. Males were unable to reproduce when plated individually, but they may have successfully developed to reproductive maturity. In Experiment 3, males were rare for N2 ($n = 0$ of 120), JU2132 ($n = 0$ of 120), and JU1726 ($n = 2$ of 120), but males appeared frequently in the CB4856 genotype ($n = 35$ of 120). Therefore, we ran two sets of analyses: one where males were censored and one where males were scored as successful dispersers.

We fit binomial-response GLMs with treatment, dose (Experiment 2), or host genotype (Experiment 3) and any interactions as predictors of variation in the proportion of dauers per replicate population that succeeded in reproducing postdispersal. We implemented the bias-reduction method of Firth (1993) in the brglm package (Kosmidis 2021; Kosmidis and Firth 2021) because of the rarity of success in exposed populations.

Table 1. Effect of parasite exposure on survival. Results of the survival assay, presented as median survival time in days from hatching for four host genotypes in the absence and presence of parasites.

Host Genotype	Median Survival Time (95% CI)	
	Control	Exposed
CB4856	15 (14, 17)	14 (14, 16)
JU1762	13 (12, 14)	6 (6, 7)
JU2132	14 (13, 16)	6 (6, 7)
N2	13.5 (13, 14)	6 (6, 7)

CROSS-SCALES COMPARISON

We assessed the fitness consequences of parasite exposure across scales by calculating the relative performance of exposed and control hosts as measured by lifetime fecundity, population growth, and dispersal success. We did not include survival in this comparison given its minimal contribution to variation in fitness (see *Results*). For the fecundity assay, the population growth assay, and Dispersal Experiment 3, we calculated the mean fitness of exposed and control hosts of a given genotype by averaging across experimental replicates. For lifetime fecundity and population growth, we calculated each host genotype's relative performance in the presence of parasites by dividing the mean number of offspring or the mean population size, respectively, of exposed hosts by that of control hosts. We also multiplied mean population sizes of exposed and control hosts by their mean dispersal success rates from Dispersal Experiment 3 to estimate the relative number of dispersers predicted to be capable of successfully colonizing a new habitat. This approach assumes that the size of the disperser pool increases with local population size and that the rate of dauer production does not change with parasite exposure. We propagated standard errors throughout these calculations.

Results

We evaluated the natural parasite *N. parisii* as a source of selection on the host *C. elegans* at the level of the patch and the metapopulation by quantifying parasite-mediated reductions in survival, fecundity, population growth (within-patch), and dispersal success (between-patch).

SURVIVAL

First, we examined the effect of parasites on host survival. Parasite exposure reduced host survival in a genotype-specific manner (Fig. S2; Table S3). Exposure did not reduce survival of CB4856 hosts but halved median survival time in the other three genotypes (Table 1). For N2 hosts, exposure reduced the probability of surviving through day 5 to $79.2 \pm 8.3\%$ (standard error), whereas no other genotype suffered mortality prior to day 6.
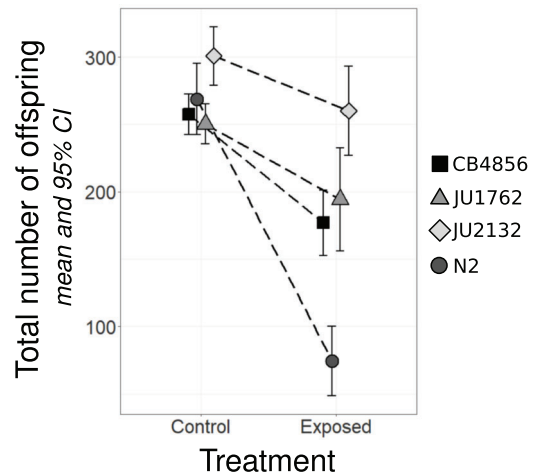
Figure 1. Effect of parasite exposure on lifetime fecundity. Results of the fecundity assay, presented as mean number of offspring, with 95% confidence intervals, for each host genotype in control and exposed conditions. Points summarize data for ~25 hosts.

FECUNDITY

Next, we quantified host fecundity in the presence and absence of parasites. On average, exposed hosts produced $34.9\% \pm 13.0\%$ fewer offspring than control hosts. The effect of exposure varied with host genotype (Table S4): reductions in fecundity ranged from 13.5% in JU2132 (300.8 ± 10.5 offspring in the control treatment; 260.2 ± 16.1 when exposed) to 72.3% in N2 (268.8 ± 12.7 in control vs. 74.4 ± 12.5 when exposed) (Fig. 1).

The timing of reproduction also contributes to variation in fitness, because early reproducers can outcompete late reproducers (Hodgkin and Barnes 1991; Feistel et al. 2019). We accordingly tested for effects on the schedule of reproduction using daily fecundity counts. Parasite exposure delayed host reproduction (Fig. S3; Table S5). In the control treatment, fecundity peaked on day 3 and then declined, with only $1.4\% \pm 0.5\%$ of offspring produced after day 4. Exposed hosts produced relatively fewer offspring on day 3 and relatively more offspring on days 5–7: $8.4\% \pm 2.9\%$ of offspring were produced after day 4. Regardless, both control and exposed hosts produced the vast majority of their offspring ($97.9\% \pm 1.0\%$) within 5 days (Fig. S3), which is before parasite exposure begins to impact host survival (Fig. S2).

POPULATION GROWTH

We then measured the effect of parasites on population growth. Consistent with the fecundity results above, parasite exposure substantially reduced the growth of host populations: at day 6, exposed populations were on average $53.1\% \pm 5.0\%$ smaller than control populations. The effect of exposure varied with host genotype (Table S6): reductions in population size at day 6 ranged
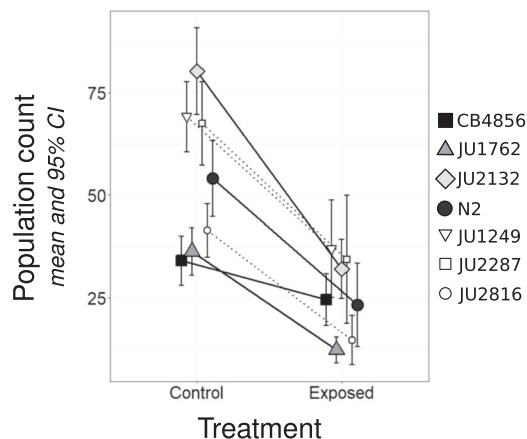
Figure 2. Effect of parasite exposure on population growth. Results of the population growth assay, presented as mean population counts, with 95% confidence intervals, of populations established by control or exposed hermaphrodites of seven genotypes and allowed to proliferate for 6 days. The four host genotypes with fecundity and survival data are represented with larger gray points, whereas the three additional genotypes are shown in white. Points summarize data for 20 replicate populations, except for JU2287, which is represented by 10 replicate populations. Population counts were obtained by counting the number of hosts in six 20-μL aliquots from a total volume of 14.5 mL per population. Further detail in Figure S4.

from 27.9% ± 10.8% (CB4856) to 66.4% ± 4.9% (JU1762) (Figs. 2, S4).

DISPERSAL

We then tested if parasites reduced dispersal success, quantified as the fraction of dauer hosts that resumed normal development and reproduced following dispersal. To this end, we performed three separate dispersal experiments. In Experiment 1, we exposed populations of N2 hosts to high parasite doses and monitored dauer larvae isolated from these populations. Exposure strongly reduced dispersal success (Table S7). All dauers from control populations recovered and reproduced ($n = 39$ of 39); only one dauer from exposed populations did ($n = 1$ of 58) (Fig. 3a).

In Experiment 2, we asked if the parasite-mediated reduction in dispersal success seen in Experiment 1 persisted at lower exposure doses. We varied timing of parasite exposure to generate variation in parasite dose experienced by larvae prior to dauer formation: for example, we inoculated host populations 6 days after their initiation to generate lower exposure doses. We again saw that parasite exposure strongly reduced dispersal success (Table S8). All dauers from control populations recovered and reproduced ($n = 178$ of 178), whereas only 32 of 179 dauer from exposed populations did (17.8% ± 7.8%). Dispersal success varied with parasite dose (Table S8), with dauers more likely to suc-

cessfully disperse from populations with low (33.3% ± 10.9%) versus high (8.4% ± 3.3%) doses (Fig. 3b).

Experiments 1 and 2 used the relatively susceptible host genotype N2, so in Experiment 3 we asked if the parasite-mediated reduction in dispersal was present for other host genotypes. Parasite exposure strongly reduced dispersal success regardless of host genotype (Table S9). Most dauers from control populations recovered and reproduced ($n = 222/225$), whereas only 20 of 210 dauers from exposed populations did (13.5% ± 8.6% across genotypes). Host genotypes differed in their success rates: 38.4% ± 18.4% of exposed CB4856 dauers were successful, whereas no exposed JU2132 dauers were (Fig. 3c). Our results held when we re-ran analyses to include male dauers, which were common in CB4856 populations (Fig. S5; Table S10).

CROSS-SCALES COMPARISON

We then estimated the multiscale effect of parasites by comparing the performance of exposed versus control hosts across within-patch and between-patch fitness components (Fig. 4). Parasite-mediated reductions in dispersal success magnified the fitness costs of parasitism estimated from within-patch metrics alone. Relative to fecundity and population growth effects, incorporating the effects of parasites on dispersal further reduced mean host performance 2.6- and 1.7-fold, respectively. Notably, for the host genotype JU2132, parasite exposure weakly or moderately reduced host fitness as measured by fecundity and population growth but eliminated it entirely after accounting for the dispersal stage. The effects of parasites on the dispersal stage also magnified the fitness advantage of resistance: performance of the relatively resistant genotype CB4856 exceeded the mean of the other genotypes by only 1.9-fold when based on population growth, but by 13-fold when accounting for the dispersal stage. These results held when including male dauers (Fig. S6).

Discussion

When modeling host-parasite interactions in metapopulations, dispersal is often simplified to an instantaneous process in which dispersers mirror their population of origin. We challenged this assumption by testing if parasites have distinct effects on dispersing hosts. We experimentally determined the effect of the natural parasite *N. parisii* on its host *C. elegans* within and between patches. Exposed hosts incurred costs of parasitism in all measured fitness components, but parasitism imposed particularly high costs at the dispersal stage. Therefore, accounting for the dispersal stage increased the estimated strength of parasite-mediated selection and the relative benefits of parasite resistance.

We found clear evidence that *N. parisii* reduces the growth rate of host populations within a patch (Fig. 2), consistent with
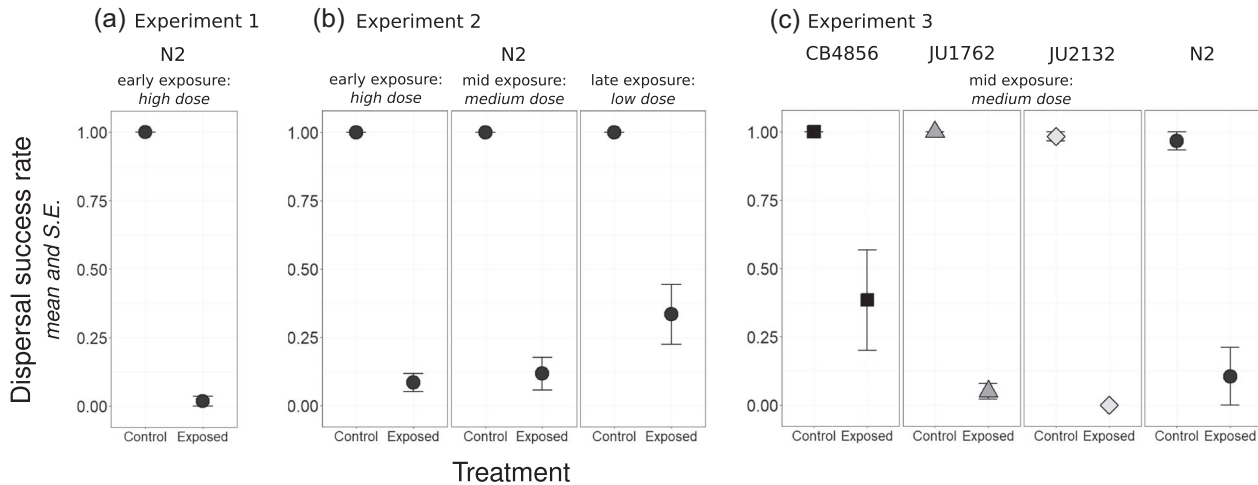
Figure 3. Effect of parasite exposure on dispersal success. Dispersal success rate is defined as the fraction of dauers that recovered from the dispersal stage and reproduced. See Figure S1 for a summary of the design of these three dispersal experiments. **(a)** Results from Dispersal Experiment 1, with N2 hosts exposed at high doses. Means and standard error bars summarize data from two control populations and three exposed populations with 20 dauers from each. **(b)** Results from Dispersal Experiment 2, with N2 hosts exposed at high, medium, and low doses as determined by the timing of parasite exposure (early, mid, and late, respectively). Means and standard error bars summarize data from three replicate populations with 20 dauers from each. **(c)** Results from Dispersal Experiment 3, with four host genotypes exposed at a medium dose. Means and standard error bars summarize data from three replicate populations with 20 dauers from each. Data shown for hermaphrodites only; see Figure S5 for data with males, which were present in CB4856 populations.
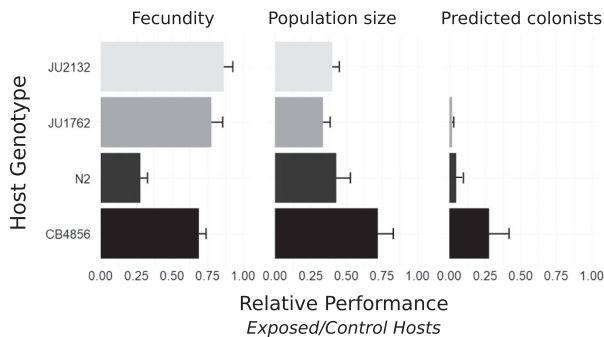
Figure 4. Comparing the effect of parasites on fitness components across scales. Includes transformed data from the fecundity assay, the population growth assay, and Dispersal Experiment 3. Bars show the ratios of the mean fitness of exposed versus control hosts as measured by fecundity, population size (within-patch), and predicted colonization success (between-patch), with standard errors, for the four focal host genotypes. To estimate relative colonization success following dispersal, we calculated the predicted ratio of successful colonists based upon the size of exposed and control populations and the rate at which dauers successfully dispersed in Dispersal Experiment 3. Data are shown for hermaphrodites only (see Figure S6 for data with males).

prior findings (Balla et al. 2015; Richaud et al. 2018). This reduction does not stem from parasite-mediated reductions in survival because *N. parisii* exposure reduces *C. elegans* survival only after reproduction is largely finished (Figs. S2, S3) (as in Troemel et al. 2008). Instead, reductions in population growth rate stem in part

from reductions in lifetime fecundity following parasite exposure (Fig. 1). However, reductions in fecundity did not directly parallel reductions in population growth; the host genotypes JU2132 and JU1762 showed relatively minor reductions in fecundity when exposed, but relatively large reductions in population size at day 6 (Fig. 1 vs. Fig. 2). We propose two hypotheses to explain the discrepancy between fecundity and population growth effects. First, genotypes may vary in transgenerational resistance. Willis et al. (2021) showed that offspring of exposed N2 mothers had much higher resistance to *N. parisii* than offspring of healthy mothers. Because our population growth assay allowed for two to three generations of reproduction, variation in transgenerational resistance could generate variation in population growth. Second, host genotypes may vary in the degree to which they promote parasite transmission, creating variation in parasite exposure over the course of population growth: for example, exposure rates may have been higher for genotypes that can reach higher densities (e.g., JU2132). Such dynamics are missed in individual fecundity experiments in which host density and parasite transmission are controlled.

We consistently found that exposure to *N. parisii* reduced host fitness even more strongly at the between-patch level, all but eliminating fitness via dispersal under a wide range of experimental conditions. *Nematocida parisii* reduced *C. elegans* dispersal success by 62%–100% (Fig. 3). Although a strong effect, this may well be an underestimate of the effect of parasites on dispersers. Dispersal is a complex process with multiple

failure points. We estimated one failure point, the developmental transition out of the dispersal stage. This transition is considered to represent the major dispersal cost in passive dispersers like *C. elegans* (Bonte et al. 2012), but parasites may also inhibit entry into the dauer stage, reduce the questing behavior required to initiate dispersal via an invertebrate vector, and/or increase mortality during transit.

Our cross-scale comparison demonstrates that the net effect of parasites on hosts—and by extension the strength of selection for resistance—is underestimated if we ignore the dispersal stage (Fig. 4). Moreover, the relative fitness advantage of genotypes changed in magnitude and rank order as we move from a narrow to a full assessment of the host life cycle. The cross-scale perspective also emphasizes the substantial bottleneck imposed by parasites at the dispersal stage, a frequent and critical stage for persistence of *C. elegans* lineages. Such strong selection may shape host traits, such as dispersal strategy, and parasite traits, such as virulence. Additionally, the interaction between *N. parisii* and *C. elegans* at the dispersal stage has epidemiological and co-evolutionary implications: heavy culling of infected dispersers could reduce parasite transmission at the metapopulation level (Hall et al. 2014; Johns and Shaw 2016) and disrupt the potential for long-term coevolution (Ladle et al. 1993). Assessment of these epidemiological and coevolutionary implications requires measurement of the degree to which successful and unsuccessful dispersers can transmit infection in new patches.

Our experiments demonstrate that the between-patch effects of parasites do not simply mirror within-patch effects. Our data suggest that, for *C. elegans*, the dispersal stage is one of the primary stages at which parasites impose selection. Incorporating the dispersal stage allows us to better predict the strength of selection across the host life cycle, the relative fitness of different host genotypes, and the evolution of parasite resistance. We expect these results to generalize to other taxa for which dispersal is frequent and critical.

AUTHOR CONTRIBUTIONS

LTB contributed to interpretation of results and wrote and edited the manuscript. ANJ designed and performed assays, collected data, and contributed to data analysis. EMT designed assays and contributed to data collection. AKG conceived and directed the study, designed and performed assays, collected and analyzed data, and wrote and edited the manuscript.

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CONFLICT OF INTEREST

DATA ARCHIVING

LITERATURE CITED

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1: Schematic of the design for the three dispersal experiments.
Figure S2: Effect of parasite exposure on survival probability.
Figure S3: Effect of parasite exposure on daily fecundity.
Figure S4: Effect of parasite exposure on population growth, by genotype.
Figure S5: Effect of parasite exposure on dispersal success; Fig. 3C with males included.
Figure S6: Comparing the effect of parasites on fitness components across scales, with males.
Table S1: Summary of replication, sample size, and censored data for survival, fecundity, and population growth assays.
Table S2: Summary of replication, sample size, censored data, and males for the three dispersal experiments.
Table S3: Survival assay - summary of analyses for survival in the presence and absence of parasites.
Table S4: Fecundity assay - summary of analyses for lifetime fecundity in the presence and absence of parasites.
Table S5: Fecundity assay - summary of analyses for daily fecundity in the presence and absence of parasites.
Table S6: Population growth assay - summary of analyses for population size in the presence and absence of parasites.
Table S7: Dispersal Experiment 1 - summary of analyses on dispersal success in the presence and absence of parasites.
Table S8: Dispersal Experiment 2 - summary of analyses for dispersal success with variation in parasite dose.
Table S9: Dispersal Experiment 3 - summary of analyses for dispersal success with multiple host genotypes.
Table S10: Dispersal Experiment 3 - summary of analyses for dispersal success with multiple host genotypes, males included.