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RESEARCH ARTICLE

Trade-offs in defence to pathogen species revealed in expanding nematode populations

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Abstract

Many host organisms live in polymicrobial environments and must respond to a diversity of pathogens. The degree to which host defences towards one pathogen species affect susceptibility to others is unclear. We used a panel of *Caenorhabditis elegans* nematode isolates to test for natural genetic variation in fitness costs of immune upregulation and pathogen damage, as well as for trade-offs in defence against two pathogen species, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. We examined the fitness impacts of transient pathogen exposure (pathogen damage and immune upregulation) or exposure to heat-killed culture (immune upregulation only) by measuring host population sizes, which allowed us to simultaneously capture changes in reproductive output, developmental time and survival. We found significant decreases in population sizes for hosts exposed to live versus heat-killed *S. aureus* and found increased reproductive output after live *P. aeruginosa* exposure, compared with the corresponding heat-killed challenge. Nematode isolates with relatively higher population sizes after live *P. aeruginosa* infection produced fewer offspring after live *S. aureus* challenge. These findings reveal that wild *C. elegans* genotypes display a trade-off in defences against two distinct pathogen species that are evident in subsequent generations.

KEYWORDS
C. elegans, defences, host-pathogen interactions, life-history evolution, reproduction, trade-offs

1 | INTRODUCTION

Hosts have evolved a variety of defence strategies due to the negative impacts of pathogens on host fitness. Hosts can avoid pathogens, limit their within-host growth (i.e. resistance) and/or limit their damage (i.e. tolerance) (Rivas et al., 2014). Within these broad defence categories, host responses to pathogens can vary from general to specific through a variety of recognition mechanisms, behaviours, signalling pathways and effector molecules (reviewed in Medzhitov & Janeway, 1997). Pathogen exposures over evolutionary time shape host–immune responses and can have consequences for host responses to other pathogens (Ezenwa et al., 2021). Cross-resistance emerges when resistance to one pathogen species results in resistance against another (Fellowes et al., 1999; Martins et al., 2014).

Conversely, trade-offs in defence can exist when improved immunity to one class of pathogens leads to increased susceptibility to another class (Marsh et al., 2011). This concept has been studied across diverse species. Examples include biotrophic and necrotrophic pathogens in plants (Spoel & Dong, 2008), tolerance to a bacterial pathogen trading off with sensitivity to a fungal pathogen in nematodes (Marsh et al., 2011) and trade-offs between resistance to bacterial species in fruit flies (Ayres & Schneider, 2008). The extent to which these immunological trade-offs between pathogen classes vary across host genotypes remains to be elucidated. The potential for host adaptation to one pathogen impacting infection outcomes of others is critical to understand how hosts evolve in natural ecosystems among a community of pathogens (e.g. Betts et al., 2018).

The nematode *Caenorhabditis elegans* has been used to study host–pathogen interactions across various microbes of clinical and natural relevance (reviewed in Sifri et al., 2005). These animals have distinct responses within classes of microbes (Wong et al., 2007) and specificity down to the pathogen strain level (reviewed in Pees et al., 2016). Most *C. elegans* research is conducted with the canonical wildtype strain N2, which is lab-adapted (Sterken et al., 2015). However, significant natural variation in the response (i.e. behaviour, morbidity and mortality) of wild *C. elegans* isolates exists against various bacterial pathogen species (Schulenburg & Ewbank, 2004; Schulenburg & Müller, 2004) and population growth can similarly vary upon infection (Petersen et al., 2015). The array of defence responses across natural host genotypes can be used as a model to make broader conclusions about the connection between immunity to different pathogen species and host fitness from an ecological and evolutionary perspective (Gasch et al., 2016).

In this study, we measured population expansion to investigate the fitness effects of a potential trade-off in host immunocompetence to two pathogen species. We exposed a panel of *C. elegans* isolates (lab-adapted N2 and nine wild genotypes) to *Staphylococcus aureus* or *Pseudomonas aeruginosa* (live and heat-killed) transiently to mimic the likely natural settings of *C. elegans*. The boom-and-bust life cycle of these nematodes suggests that transient exposure to patches of microbes, including pathogens, are more common than constant exposure to the same microbe (Frézal & Félix, 2015). Exposures were also timed to ensure immune upregulation before reproduction, and we have previously found that brief pathogen exposures to *S. aureus* during this period has delayed reproductive output (Ordovas-Montanes et al., 2022). We then measured the resulting host population size, two generations later, derived from the exposed parental founder worm. Quantifying the host population size over a fixed time concurrently accounts for the lethal and non-lethal impacts of infection on a variety of host life-history traits, including reproductive rate, developmental time and survival (Feistel et al., 2019).

Although the two pathogen strains and species used in our study are both novel to *C. elegans*, *Staphylococcus* and *Pseudomonas* (Grewal & Richardson, 1991) genera have been found present in mushroom environments where *C. elegans* can occur. The latter are prominent microbes in the natural compost environment of the nematodes (Carrasco et al., 2020; Samuel et al., 2016), and Proteobacteria (including the Pseudomonadaceae family) are abundant in the native *C. elegans* microbiome (Dirksen et al., 2016). The selected pathogen species also differ in their infection mechanisms and elicit distinct host responses in ways that indicate specificity (Irazoqui et al., 2010). In particular, Irazoqui et al. (2010) found that 10 specific genes were upregulated similarly by hosts against live and heat-killed *S. aureus*, and 10 *P. aeruginosa*-specific genes were highly upregulated against live pathogens only. These findings allowed us to disentangle the fitness costs of *S. aureus*-induced immune upregulation and pathogen damage and use heat-killing as negative controls (for *P. aeruginosa*). Across our diverse panel of nematode isolates and pathogens, we examined whether there were (i) trade-offs in the fitness cost of hosts experiencing pathogen damage and/or immune upregulation with optimal host reproductive success across two pathogens, (ii) differences in fitness costs caused by pathogen damage and/or immune upregulation and, ultimately, (iii) trade-offs in fitness costs associated with investment towards resistance to two pathogen species.

2 | MATERIALS AND METHODS

2.1 | Nematode and bacterial strains

We used *Caenorhabditis elegans* N2 nematodes and the wild isolates CB4853, CB4854, CB4858, ED3017, JU1400, JU1490, JU258, LKC34 and QX1211. These isolates were randomly chosen from across a phylogenetic tree (Andersen et al., 2012) to represent a wide panel of nematodes from various geographical locations and genetic backgrounds (Table S1).

Nematode populations were maintained at 20°C on nematode growth medium (NGM) with *Bacillus subtilis* PY79 or *Escherichia coli* OP50 before being exposed to the pathogens *Staphylococcus aureus* MSSA476 or *Pseudomonas aeruginosa* PA14, respectively. We ensured that nematodes were consistently exposed to Gram-positive (MSSA476 treatments paired with PY79 food) or Gram-negative (PA14 treatments paired with OP50 food) bacteria throughout the assays.

Staphylococcus aureus was grown in 3–5 ml of Todd-Hewitt Broth, whilst *P. aeruginosa* was grown in 3–5 ml of Luria broth (LB), and food sources were grown in 13–15 ml aliquots of LB. NGM plates were seeded with 800 µl of bacterial food diluted to OD_{630} = 0.15. For the Gram-positive assays, 55 mm Tryptic Soy Agar (TSA) plates were seeded with 60 µl of the exposure strain diluted to OD_{630} = 0.15. We used 55 mm NGM plates (also seeded with 60 µl) for Gram-negative assays, to have slow-killing nematode exposures as opposed to fast-killing, where nematodes die within hours and would be unable to reproduce (Tan et al., 1999). All maintenance and exposure plates, without worms, were incubated at 30°C overnight.

For exposures involving heat-killed bacteria, overnight cultures of bacteria were first diluted to OD_{630} = 0.15, then 1 ml transferred to microcentrifuge tubes and incubated in a water

bath set to 88°C for 1 h. Incubation conditions were determined upon surveying the literature and trialling a range of temperatures. We incubated bacteria at temperatures that were lethal for them and no growth was detected. Heat-killed samples were allowed to cool and then plated as described above for 55 mm TSA and NGM exposure plates.

2.2 | Egg preparation and synchronization

Before each experiment, starved nematodes from maintenance plates were transferred to new 90 mm NGM plates with bacterial food by taking a small agar chunk, one for each host isolate. Three days later, each plate was washed with M9 and Triton-X (0.01%), and all gravid nematodes and eggs transferred to a 15 ml conical tube. We bleached the nematodes according to standard protocols (Stiernagle, 2006). The following day, the resulting synchronized larval stage 1 (L1) nematodes were plated on 90 mm NGM maintenance plates to a density of about 1800 nematodes per plate for each isolate. All nematodes were reared at 20°C for 44 h, until they reached L3/L4.

2.3 | Transient exposure of nematode hosts to bacterial pathogens

Nematodes were exposed to either food or bacterial pathogen (heat-killed or live) at L3/L4 stage, around 44 h post-L1 synchronization. To expose nematodes to bacteria, nematodes were washed off NGM maintenance plates with M9 + Triton-X into microcentrifuge tubes and then washed three times by allowing nematodes to pellet by gravity and be re-suspended. Approximately, 100 nematodes were transferred to one of six replicate plates with a spread of either pathogen or food (Figure 1). This time was noted as 0 h post-L4. Exposure plates were incubated at 25°C for 8 h, the point at which nematodes have expressed immune genes specific to each pathogen but have not yet started to lay eggs (Aprison & Ruvinsky, 2014; Irazoqui et al., 2010). The exposure timeframe was chosen to enhance the immune-upregulation effects leading up to the host reproductive period.

2.4 | Population growth assays

To measure the impact of transient pathogen exposure on population growth, the number of individuals in a single host lineage across three generations (P, F1 and F2) was counted. The lineage expansion assay method was followed as described in Feistel et al. (2019), with a few modifications. This metric allows us to look at pathogen effects beyond the lifetime of a single host. Changes to host population sizes indicate exposure-mediated effects on a host isolate that encompass host reproductive rate, developmental time and survival concurrently. After 8 h exposure to either pathogen or control food, four founder nematodes were picked off each exposure plate onto four separate 90 mm NGM plates seeded with bacterial food. Plates were left at 25°C for 5 days whilst nematodes produced F1 and F2 generations. On the final day, each plate was washed with M9 + Triton-X until most nematodes were displaced. The suspension was diluted to a total volume of 4 ml in a conical tube, and then six 10 µl droplets were sampled and counted to determine the

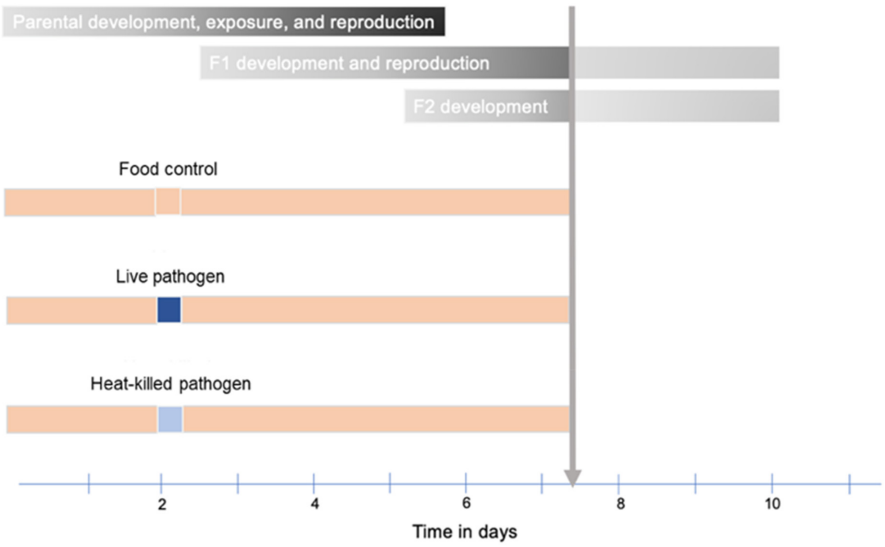

FIGURE 1 Experimental method for host lineage expansion assays to measure population sizes. Experiments were performed across 10 isolates. Parental nematodes developed on *Bacillus subtilis* PY79 or *Escherichia coli* OP50 control food types for 2 days. Nematodes were then transferred to another control food plate or exposed to live or heat-killed pathogen (*Staphylococcus aureus* or *Pseudomonas aeruginosa*) for 8 h. After transfer to control food for the duration of the experiment, the populations were allowed to expand for 5 days. Colour changes indicate transfer of nematodes between plates. Grey gradients indicate progression in development. Grey arrow indicates the final time-point at which nematodes were washed off plates and counted to determine population size

population size for each plate. Treatments consisted of three biological replicates per assay, and each experiment was repeated twice to have six replicate plates for a given bacterial exposure. Overall, we set up six replicate plates × four founder worm replicates × ten host isolates × three bacterial treatments × two pathogen types for a total of 1440 tracked founder worms. We censored a small number of worms that left the plate or were injured/killed after picking (Tables S2 and S3).

2.5 | Statistical analyses

Data were analysed in R version 3.6.1 and RStudio version 1.2.1335 (RStudio Team, 2019). To calculate the population size from a founder worm, the average was taken from six 10 µl droplets and back-calculated to give the number of worms per plate. Population sizes from four replicate worms from an exposure plate were combined to give a mean population size for that plate (Figures S1 and S2). A mean of those six plates was calculated to give a mean population size for a given host isolate for a specific bacterial exposure.

To determine the correlation between pathogen-exposed host population sizes and control host population sizes, we used Pearson's product–moment correlation and adjusted for multiple testing with false discovery rate correction. This correlation test was done for each pathogen treatment compared with food controls (Figures S3 and S4). These plots indicate that some host genotypes have consistently high or consistently low population sizes (independent of the exposure); therefore, we employed ratios to compare fitness costs across host isolates. For subsequent analyses, count data were transformed into fitness cost ratios of mean population size on pathogen (either live or heat-killed) over mean population size on control food, matched by batch identity (Figures S5 and S6). The ratios represent a proxy for immune competence since host defences are mechanisms employed to minimize fitness loss upon infection (Schmid-Hempel, 2013). We used Spearman's rank correlation tests to determine the relationship between relative population size after transient pathogen exposure and food control population size, as well as the relationship of relative population sizes of host genotypes exposed to *S. aureus* or *P. aeruginosa*.

Differences between live and heat-killed bacterial treatments, for both *S. aureus* and *P. aeruginosa*, were examined using generalized linear models with quasibinomial error distribution as the data were over-dispersed. We tested for differences between live and heat-killed exposures for a given bacterial species using analysis of variance to test for main effects by host isolate, bacterial exposure, and the interaction of the two, for both *S. aureus* and *P. aeruginosa* data sets using the car (Fox & Weisberg, 2019) and multcomp (Hothorn et al., 2015) R packages. To quantify fitness reductions and fitness boosts between live and heat-killed exposures, we used the percentage difference formula from Cole and Altman (2017) to compare mean relative population sizes after each pathogenic treatment.

Data figures were made using the ggplot2 (Wickham, 2016) and RColorBrewer (Neuwirth, 2014) packages in R.

3 | RESULTS

3.1 | Effect of transient *S. aureus* exposure on host population growth

We investigated the effect of transient *S. aureus* exposure on the population sizes of one lab-adapted and nine wild nematode isolates. The population sizes after pathogenic exposure were generally lower than population sizes from the control food treatment, which is evident in seeing points below the $y = x$ line in Figure S3. We found that reproduction after pathogen exposure to live (Figure S3A: Pearson's product–moment correlation: $t = 4.92$, $df = 8$, $p = 0.001$, $r = 0.87$) or heat-killed (Figure S3B: Pearson's product–moment correlation: $t = 7.48$, $df = 8$, $p = 0.0001$, $r = 0.94$) *S. aureus* correlated with reproduction on food. Isolates that performed well on food also performed well after pathogen exposure, relative to other isolates (Table S4). Because genotypes tended to be 'high performing' or 'low performing', we analysed subsequent data as relative population sizes to better compare fitness cost magnitudes across genotypes.

We then investigated the distinct costs of immune upregulation versus pathogen damage to host population growth. We found a significant difference between live versus heat-killed *S. aureus* exposure (Figure 2: Quasibinomial GLM, $\chi^2 = 4.66$, $df = 1$, $p = 0.031$): hosts exposed to live *S. aureus* (versus heat-killed) experienced greater fitness costs compared with their control-treatment population sizes. However, there was no difference in population sizes between host genotypes within each treatment (Figure 2: Quasibinomial GLM, $\chi^2 = 1.71$, $df = 9$, $p = 0.995$). One aspect to note is the larger variation in the relative population sizes for the heat-killed treatments (Figure S7). Across host genotypes, there was an 18% reduction in relative population sizes on live *S. aureus* (average rel. pop. size = 0.82) compared with heat-killed *S. aureus* (average rel. pop. size = 0.98). This decrease is associated solely with live pathogen damage, and not the immune upregulation that hosts in both treatments experience (Table S5). Notably, the lab-adapted N2 strain exhibited the biggest loss in relative population size (33.26%) from heat-killed to live and thus suffered the largest fitness cost due to pathogen damage compared with other strains.

We examined whether there was an evolutionary cost of immunocompetence by comparing the relative population sizes (ratio metric indicating fitness cost after pathogen exposure) to population sizes on control food. We found no evidence of a trade-off between the immune and reproductive traits after either live (Figure 3a: Spearman's rank correlation, $p = 0.4$, rho = −0.3) or heat-killed (Figure 3b: Spearman's rank correlation, $p = 0.4$, rho = 0.4) *S. aureus* exposure. The population size versus immunocompetence comparison for the lab-adapted N2 across the treatments was on the intermediate-to-low end, compared with the range of wild isolate data.

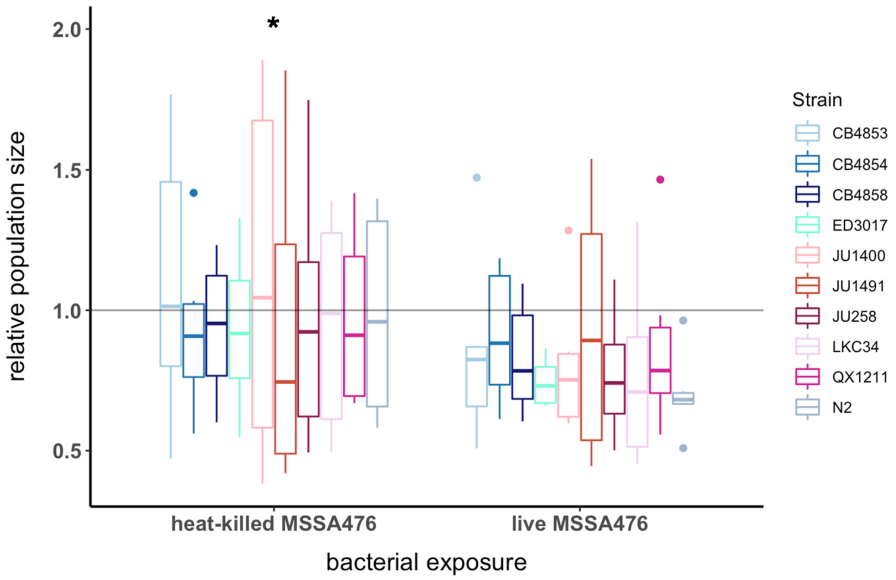

FIGURE 2 Relative population sizes for ten nematode isolates after transient exposure to live and heat-killed *S. aureus* (MSSA476). Values are relative to population sizes on *B. subtilis* food. Boxes show first quartile, median and third quartile of the data, whiskers extend 1.5 times the interquartile range from the first and third quartiles, and circles indicate outliers. Grey line at $y = 1$ represents no difference between host population size on *S. aureus* treatment and control food. Host genotypes vary by colour. $^*p < 0.05$

3.2 | Effect of transient *P. aeruginosa* exposure on host population growth

We next investigated the effect of transient *P. aeruginosa* exposure on nematode host population size. In both live and heat-killed pathogen exposure treatments, we found that nematode population sizes were correlated positively with population sizes on food (Figure S4A: *P. aeruginosa* live vs. food: Pearson's product–moment correlation: $t = 2.85$, $df = 8$, $p = 0.02$, $r = 0.71$; Figure S4B: *P. aeruginosa* heat-killed vs. food: $t = 4.18$, $df = 8$, $p = 0.006$, $r = 0.83$). The trends with host isolate performance on Gram-negative bacteria (Table S6) were not as clear as with the aforementioned trends for hosts on Gram-positive bacteria (Table S4).

Nematodes had significantly higher population sizes after live *P. aeruginosa* exposure compared with heat-killed exposure (Figure 4: Quasibinomial GLM, $\chi^2 = 24.323$, $df = 1$, $p < 0.0001$), both relative to food. Also, we found significant differences among the host isolates (Figure 4: Quasibinomial GLM, $\chi^2 = 20.51$, $df = 9$, $p = 0.015$) indicating that some nematode isolates increased their reproduction more than others. There was no significant interaction between bacterial exposure and host isolate (Figure 4: Quasibinomial GLM, $\chi^2 = 9.76$, $df = 9$, $p = 0.37$). Across host genotypes, there was a 20% boost in relative population sizes on live PA14 (average rel. pop. size = 1.33) compared with heat-killed PA14 (average rel. pop. size = 1.08) (Table S7). In line with the percentage difference result after Gram-positive exposures, the lab-adapted N2 strain exhibited the greatest increase in relative population size (36.47%) from heat-killed to live.

We examined whether there was an evolutionary cost of immunocompetence by comparing the relative population sizes of each host genotype to their population sizes on control food. We found no evidence of the expected negative correlation between defence and reproduction after either live (Figure 5a: Spearman's rank correlation, $p = 0.2$, rho = −0.4) or heat-killed (Figure 5b: Spearman's rank correlation, $p = 0.5$, rho = −0.3) *P. aeruginosa* exposure. The population size versus immunocompetence for the lab-adapted N2 across the treatments was on the intermediate-to-low end for heat-killed PA14 and higher for live PA14, compared with the range of wild isolate data.

3.3 | Comparisons between host responses to two opportunistic pathogens

Host isolates that had higher population sizes after exposure to *S. aureus* also had higher population sizes in the absence of the pathogen (Table S4 and Figure S3), whereas hosts on *P. aeruginosa* were not as consistent across the bacterial treatments (Table S6 and Figure S4). We aimed to see whether there was a relationship between the degree to which nematode population sizes were affected by exposure to the two opportunistic pathogen species. We found a trade-off in immunocompetence across host isolates after live opportunistic pathogen exposures (Figure 6a: Spearman's rank correlation rho = −0.72, $p = 0.02$). Hosts that performed better after *P. aeruginosa* exposure did worse after exposure to *S. aureus*. Conversely, we did not find a significant relationship among relative population sizes resulting from exposure to heat-killed pathogens (Figure 6b: rho = 0.079, $p = 0.84$). Since the control food is in the denominator of each ratio, this accounts for food-driven population differences. Compared with the wild isolates, N2 hosts performed better in terms of relative population size after live *P. aeruginosa* exposure than after *S. aureus* exposure.

4 | DISCUSSION

The long-term fitness consequences of transient exposures to different pathogen species are relatively unexplored, even though most host organisms live in multi-microbial environments with a diversity of pathogen species. In this study, we found that short exposures revealed more evidence of genetic variation in relative population sizes

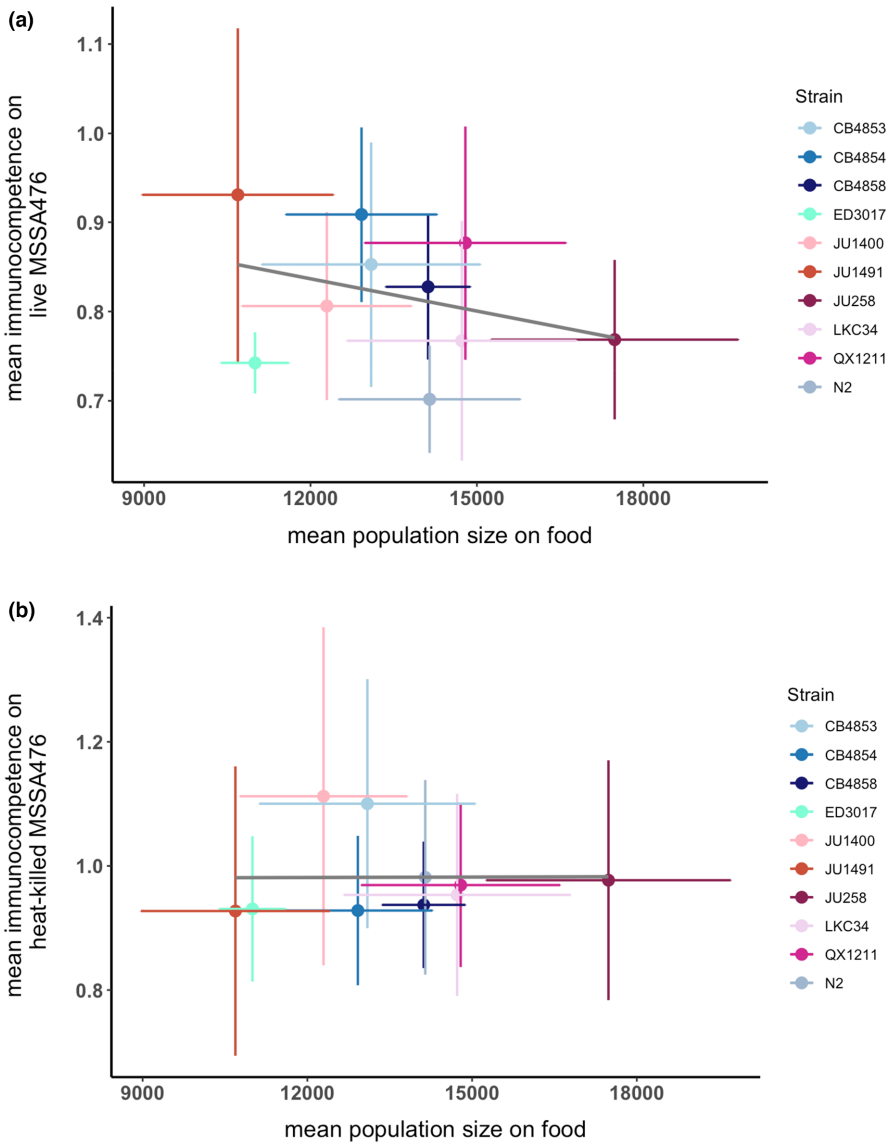

FIGURE 3 Population size of each nematode isolate on food (mean ± 1 *SE*) versus mean immunocompetence (ratio of population after pathogen exposure relative to population on food ± 1 *SE*) after exposure to either (a) live or (b) heat-killed *Staphylococcus aureus* across 10 host isolates. Host genotypes vary by colour. Grey line depicts linear regression of immunocompetence on population size on food

in response to *P. aeruginosa* exposure than to *S. aureus*. This contrast may emerge from the nematodes having a more extensive evolutionary history with several members of the *Pseudomonas* genus as food sources, pathogens and symbionts. Pseudomonads are prominent microbes in the natural compost environment of the nematodes (Berg et al., 2016; Montalvo-Katz et al., 2013; Samuel et al., 2016) and are abundant in the native microbiome (Dirksen et al., 2016). In contrast, *C. elegans* can also encounter some *Staphylococcus* species in natural habitats (Montalvo-Katz et al., 2013; Rossouw & Korsten, 2017) and *S. aureus* can adapt to nematode genotypes after a period of pathogen experimental evolution in the lab (Ekroth et al., 2021). That said, the strain of *S. aureus* used herein was notably novel to *C. elegans*, and *Staphylococcus* species are not found as frequently *as Pseudomonas species in the C. elegans microbiome*. Thus, our results are similar to those of a recent study in wild buffalo which found resistance to helminth parasites sharing a long-term evolutionary history was traded-off with increased disease severity by a microbial infectious agent (Ezenwa et al., 2021).

Across host isolates, we found that transient exposure to live *P. aeruginosa* significantly increased population sizes relative to heat-killed bacteria. Essentially, there was a fitness benefit from short-term exposure to this pathogen. This host response could be due to pathogen-induced fecundity compensation, where hosts increase offspring production after pathogen exposure (Pike, Ford et al., 2019). Because only the parental generation was exposed to *P. aeruginosa*, they or their offspring may have recovered faster than the ones exposed to *S. aureus*, resulting in increased population sizes. Alternatively, *P. aeruginosa* may act more like a food than a pathogen when hosts are exposed transiently; it may become harmful only after longer or constant exposure. Since *P. aeruginosa* kills hosts by accumulating in the intestine in our experiments (Tan et al., 1999), more time may be necessary for the pathogen to grow in hosts. Overall, our results demonstrated that transient exposure to pathogens can still have a significant effect on host population sizes two generations later. Additional work is needed to characterize how the time period of host–microbe interactions can cause shifts between nutrition and pathogenicity. The extent to

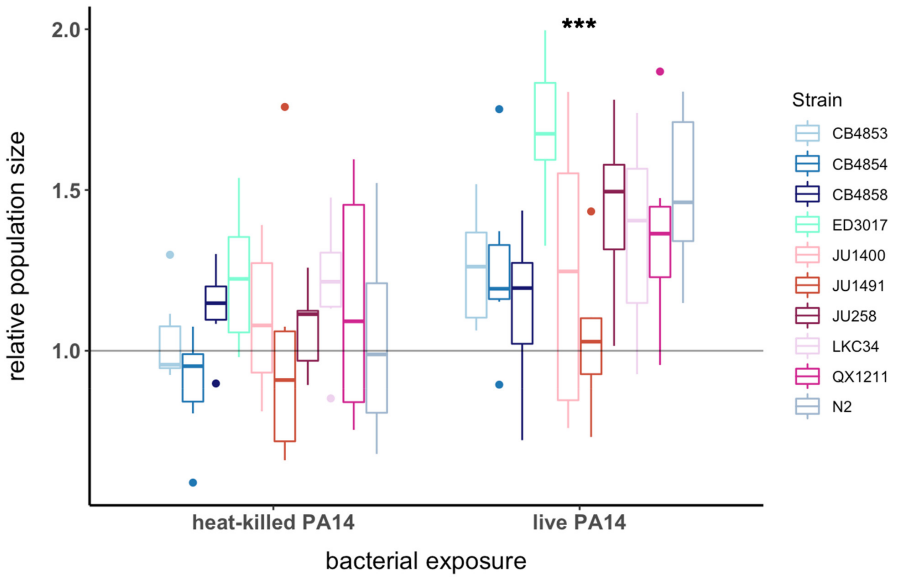

FIGURE 4 Relative population sizes for 10 nematode isolates after transient exposure to live and heat-killed *P. aeruginosa* (PA14). Values are relative to population sizes on *E. coli* food. Boxes show first quartile, median and third quartile of the data, whiskers extend 1.5 times the interquartile range from the first and third quartiles, and circles indicate outliers. Grey line at $y = 1$ represents no difference between host population size on *P. aeruginosa* treatment and control food. Host genotypes vary by colour. $^{***}p < 0.0001$

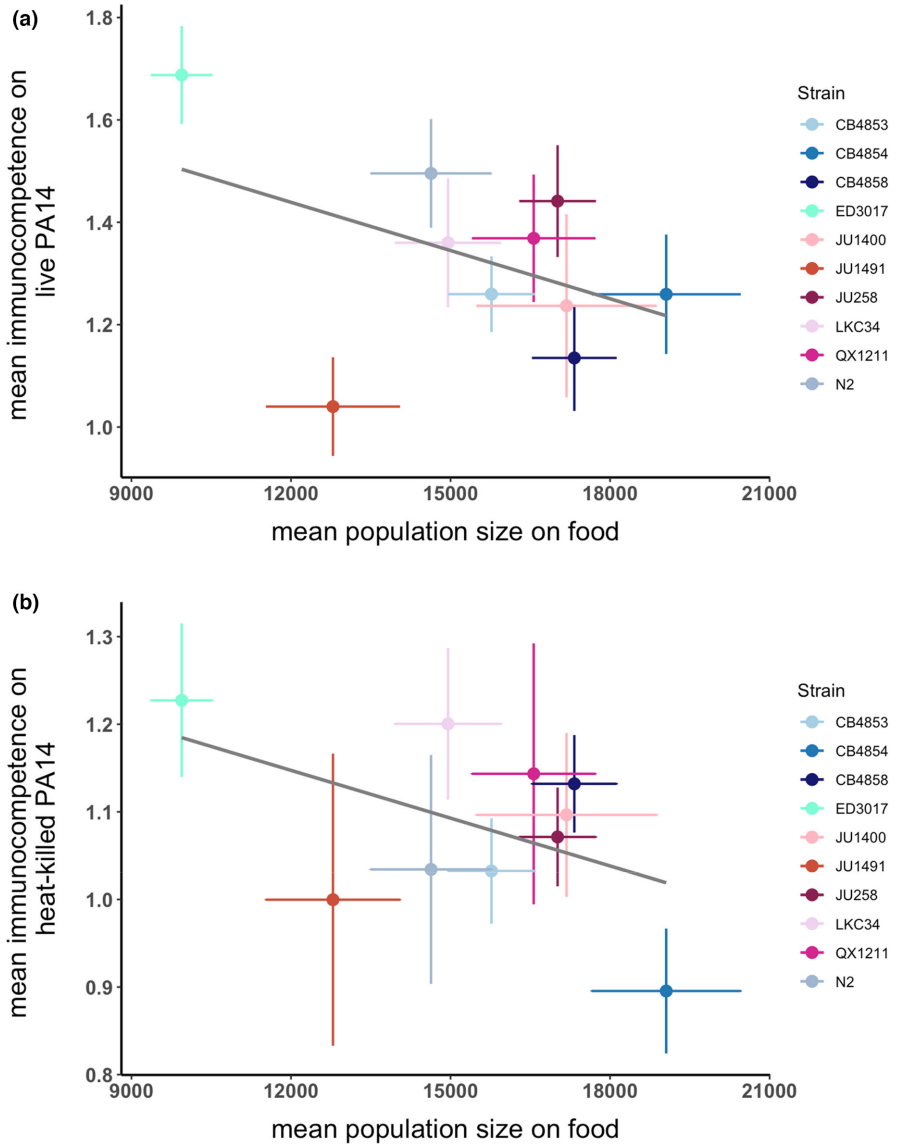

FIGURE 5 Population size of each nematode isolate on food (mean ± 1 *SE*) versus mean immunocompetence (ratio of population after pathogen exposure relative to population on food ±1 *SE*) after exposure to either (a) live or (b) heat-killed *Pseudomonas aeruginosa* PA14, across 10 host isolates. Host genotypes vary by colour. Grey line depicts linear regression of immunocompetence on population size on food

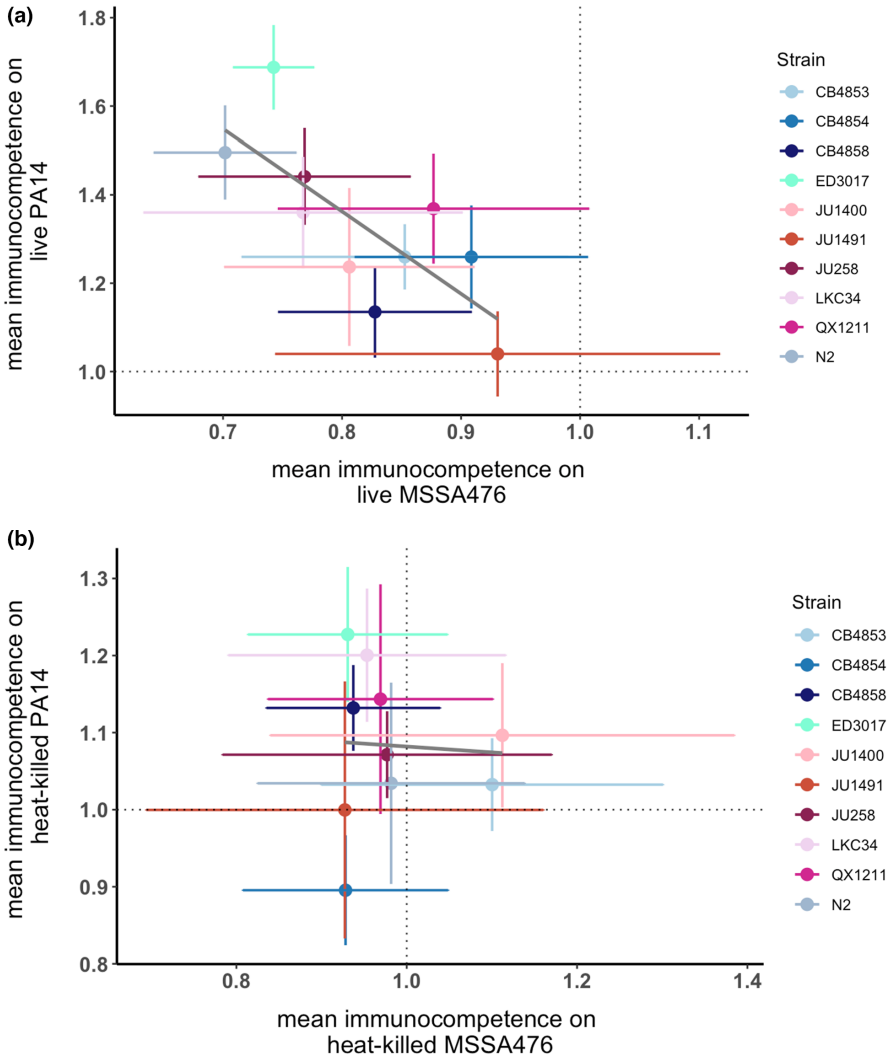

FIGURE 6 Relationship between immunocompetence across ten nematode isolates after exposure to *S. aureus* MSSA476 and *P. aeruginosa* PA14. Trade-off between population sizes after exposure to the two live pathogens (a), and no trade-off between population sizes after exposure to the two heat-killed pathogens (b). Values are relative to population sizes on food (*B. subtilis* PY79 for *S. aureus* MSSA476 exposures, *E. coli* OP50 for the *P. aeruginosa* PA14 exposures). Mean relative population sizes are shown with 1 standard error. Dotted lines at $x = 1$ and $y = 1$ represent no difference between pathogen treatment and food. Host genotypes vary by colour. Grey line depicts linear regression of immunocompetence with *P. aeruginosa* on immunocompetence with *S. aureus*

which host immunity against *P. aeruginosa* or potential nutritional benefits of microbes drive increased population growth should be disentangled.

Moreover, we did not find a trade-off between immunocompetence to pathogen and reproduction in the absence of pathogen. The exposure time was potentially not long enough. Consequences of nematode exposure to *S. aureus* have been examined under a range of timeframes, mostly longer than 8 hours (Garsin et al., 2001; King et al., 2016; Sifri et al., 2003). Perhaps a longer exposure, or an earlier-age exposure during spermatogenesis, would have had a significant impact for a host where reproduction of hermaphrodites is sperm-limited (Kimble & Ward, 1988). It remains to be determined how hosts are infected in natural settings: at what stage nematodes are most susceptible to infection (Ben-Ami, 2019) and the extent to which pathogens persist or are transient, given the nematodes' avoidance behaviour. Another possibility is that our hosts were not constrained by resources, since food depletion can sometimes reveal costs of immunity that are masked by unlimited food (McKean et al., 2008). That said, the relationship between host nutrition and infection outcomes can often be variable (Pike, Lythgoe et al. 2019) or absent (Roberts & Longdon, 2021).

Taken together, our results suggest that the genetic trade-offs in host defence between two distinct pathogens may be an important determinant of population size. This finding provides evidence that host immune responses can be calibrated by evolution in a multi-microbial environment since fitness in the presence of one pathogen may be traded-off with fitness to another.

AUTHOR CONTRIBUTIONS

M.O.M., G.M.P. and K.C.K. conceived and designed the study. M.O.M conducted the experiment, with the guidance from K.C.K. and G.M.P. M.O.M., C.R.M. and K.L.H. conducted the statistical analysis. M.O.M., K.L.H., and K.C.K. wrote the manuscript with input from all authors.

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CONFLICT OF INTEREST

There are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available in Dryad: https://doi.org/10.5061/dryad.s7h44j18s.

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