Risk taking of educated nematodes

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Abstract

Nematode parasites rely on successful host infection to perpetuate their species. Infection by individual nematode parasites can be risky, however; any one individual could be killed by the host’s immune response. Here we use a model system to show that environmental cues and parasite past experience can be used by entomopathogenic nematodes to reduce individual risk of infection. Past parasite experience can more than double the infective virulence (number of host invaders) of a given cohort of entomopathogenic nematode parasites. This plasticity in individual parasite risk-taking and associated infection can be used to manage infection of parasitic nematodes: enhancing biological control with entomopathogenic nematodes and developing behavioral and chemical strategies to reduce infection by vertebrate and plant parasitic nematodes.

Introduction

Nematode parasites—whether pathogens of vertebrates, insects, or plants—exist in dynamic systems where host infection is a critical objective [1]. While locating hosts against a backdrop of ephemeral signals is a primary challenge, nematode parasites face a secondary, and perhaps more significant challenge after arriving at their host: successful infection [1].

In a co-evolutionary arms race with nematode pathogens, their host vertebrates, insects and plants have developed a multitude of defenses to prevent exactly what the nematode parasite desires—successful infection and colonization of the host [1–3]. Indeed, this stage incurs considerable risk on the part of an individual parasitic nematode. Physical, chemical, and immune barriers all combine to prevent successful infection of the host. In vertebrate parasitic systems, Mucin and Toll receptors provide a first and second line of host defense respectively [4]. Following that gauntlet, vertebrate parasitic nematodes could then encounter a TH2 cytokine response [5, 6]. Similarly, plant parasitic nematodes encounter a multilayered defense strategy involving reactive oxygen species, production of specific secondary metabolites and fortification of cell walls [7, 8]. Insect parasitic nematodes must circumvent the insect cuticle, then will often encounter encapsulation responses [9]. Regardless of the particular defense strategy, host responses render individual infection by nematode parasites a potentially mortal risk.
Parasitic nematodes have evolved many means to address these risks in order to overcome and influence host behavior [10]. One of the most successful infection strategies involves overwhelming the host immune system through group attack [11]. This type of behavior is seen perhaps most clearly in insect parasitic nematodes, called entomopathogenic nematodes, where large numbers of pathogens are required to overcome a host’s immune system [12, 13]. Any less than the critical number and infection by too few nematode parasites results in an encapsulation response that renders a brave foray into the host an untenable and lethal outcome for the individual nematode [12, 13].

Group attack to overwhelm host responses does not necessarily obviate risk on the part of an individual entomopathogenic nematode, however. Because critical numbers are needed to achieve successful infection, any individual suffers from critically incomplete knowledge: How many parasites from their cohort will ultimately infect the same host? In a system with many decision making agents, knowledge of another’s decision can reduce an individual nematode’s risk of encapsulation [14].

Here we explore nematode parasite risk taking in the context of host infection behavior in order to understand both basic biology and the potential to manage infection in practical applications. Specifically, we address the question of whether entomopathogenic nematode infection behavior is plastic. Behavioral plasticity, learning and modifying behavior based on past experience, has been shown in relation to host-seeking and orientation behavior in entomopathogenic nematodes, but is unclear the extent to which exposure influences direct interaction with the host [15, 16]. The presence of and exposure to host plant volatiles could change entomopathogenic nematode infection behavior. Given that plant volatiles could be used to signal the presence of critical resources in the form of host availability, the hypothesis is that entomopathogenic nematodes may be more likely to infect insect hosts associated with familiar plant volatile cues.

To explore this question, we establish baseline risk profiles of infectivity, explore how environmental cues and past experience can influence that infectivity and then determine the resultant effects on host mortality. Entomopathogenic nematodes were chosen as a model study system due to their prolific nature, group dynamics, learning ability (social behavioral plasticity [15]), and use of plant volatiles as environmental cues for locating hosts [17].

Results

Infection

To establish baseline infectivity profiles of entomopathogenic nematodes in the presence or absence of common belowground plant volatiles, Galleria mellonella larvae were paired with doses of either pregeijerene or α-pinene then inoculated with 1000 infective juveniles of either Heterorhabditis indica or Steinernema diaprepesi in small arenas containing 1g of sterilized sand and the host insect. Pregeijerene is an herbivore-induced plant volatile known to recruit these species of entomopathogenic nematodes to Diaprepes abbreviatus larvae feeding on citrus roots [18, 19]. α-pinene is a common plant volatile recovered from the same system and is known to innately repel these species of entomopathogenic nematode [15, 18, 19]. Twenty-four and forty-eight hours post inoculation, larval mortality and number of infective juveniles that entered the host cadaver were assessed.

Importantly, these assays were small in size. Entomopathogenic nematodes can move relatively long distances (1,000x body length) in response to volatile cues as many previous studies have established [18, 20]. Critically distinct from recruitment behavior, however, is infection of an insect host. Just because entomopathogenic nematode infective juveniles are attracted
and recruited to the vicinity of the host insect does not mean the entomopathogenic nematodes will necessarily infect the insect.

In these assays that obviated long-distance recruitment, species, hours post-infection, volatile treatment, and first order interactions significantly explained observed numbers of infecting nematodes ($\chi^2 = 325.8, 187.6, 2684.1, >628.7; df = 1,1,5,5; P < 0.001$). *H. indica* infective juveniles entered the larval hosts at 2.1 [95% confidence interval: 2.04, 2.17] (Tukey HSD, $P < 0.001$) times the rate of *S. diaprepesi* infective juveniles. Additionally there were 2.37 [2.30, 2.45] and 2.73 [2.61, 2.86] ($P < 0.001$) times more infective juveniles inside the cadavers after 48 hours as compared to 24 hours for *H. indica* and *S. diaprepesi*, respectively (Fig 1).

Importantly, presence of any of the two plant volatiles triggered a significant ($P < 0.001$) increase in host infection by entomopathogenic nematodes over controls without plant volatiles (Fig 2, S1 Fig for raw numbers). Additionally, main effects higher doses of these volatiles resulted in higher numbers of infective juveniles recovered from cadavers for both pregeijerene and $\alpha$-pinene ($P < 0.001$).

**Infection plasticity**

To examine how past experience can change future infection behavior of parasitic nematodes, cohorts of 1000 infective juveniles were placed in glass vials containing water with or without the plant volatiles $\alpha$-pinene or pregeijerene. Forty-eight hours later, infective juveniles were
washed three times and placed into an arena containing host larvae and plant volatiles as above.

Prior exposure to a compound in glass vials significantly increased infection (30% [27%, 33%] on average; 147% [128%, 168%] for H. indica exposed to 10 ng α-pinene and 192% [173%, 313%] for S. diaprepesi exposed to 10 ng pregeijerene) when that same compound was paired with the insect larva (Fig 3, S2 Fig for raw numbers). This is true for both compounds, although exposure to pregeijerene produced significantly higher increases in infection ($P < 0.001$). These effects varied by species and volatile; species, volatile treatment, exposure status, and first order interactions significantly explained observed numbers of infecting nematodes ($\chi^2 = 68.3, 902.3, 55.2, >1081.5; df = 1,4,1,4; P < 0.001$). Interestingly, prior exposure to a compound reduced infection when that compound (pregeijerene) was not paired with the host insect (60% [57%, 62%] reduction on average in nematode only controls, $P < 0.001$) (Fig 3, S2 Fig for raw numbers).

**Host mortality and susceptibility**

To determine how infection by nematode parasites influences host mortality, we used logistic regression models to estimate the probability of host death given varying levels of infection by entomopathogenic nematodes. Both the number of nematodes infecting the host insect and the time post infection had significant effects on host mortality ($\chi^2 = 50.9, 136.9; df = 1,1; P < 0.001$). Additionally, there was a significant interaction between species and the number of infective juveniles infecting the host ($\chi^2 = 11.2; df = 1; P = 0.001$). H. indica showed higher probabilities of host mortality for lower numbers of infecting nematodes (Fig 4). Additionally, H. indica infection could result in up to 100% probability of mortality in 24 hours (Fig 4). S. diaprepesi, in contrast, only achieved significant probability of mortality after 48 hours (Fig 4).
Risk taking and infection by parasitic nematodes is influenced by the host environment and past experiences of the parasitic nematode. Host insects in the presence of the plant volatiles pregeijerene and $\alpha$-pinene can suffer an almost doubling in infection rate depending on the

![Graph showing increase in infection after exposure to treatment volatiles.](https://doi.org/10.1371/journal.pone.0205804.g003)

**Discussion**

Risk taking and infection by parasitic nematodes is influenced by the host environment and past experiences of the parasitic nematode. Host insects in the presence of the plant volatiles pregeijerene and $\alpha$-pinene can suffer an almost doubling in infection rate depending on the

![Graph showing mortality from infection.](https://doi.org/10.1371/journal.pone.0205804.g004)
species assayed (Fig 2). Likewise, prior exposure to (past experience with) those same plant volatiles further increases infection, in some cases increasing infection by more than 100% (Fig 3). Interestingly, nematode parasites previously exposed to a volatile are less likely to infect a host insect if the volatile to which they had been exposed is not present. This observation corroborates previous observations that volatile exposure confers specific preference [15]; nematode parasites prefer volatiles with which they have experience, but seem to lose interest in the absence of that volatile.

That presence of and prior exposure to plant volatiles alters infection profiles of entomopathogenic nematodes suggests that these volatiles could perhaps be functioning as cues to answer that critical question: How many compatriot parasites will ultimately infect the same host? Such volatiles could be used as a proxy for infection potential. Evolutionarily, if many nematode parasites rely on similar environmental cues, any individual parasite is more likely to successfully infect a host if one of those similar environmental cues is present. The risk to any individual is reduced.

The relationship between individual parasite risk and reward is perhaps seen most clearly in examining the probability of host mortality as a function of number of infecting nematodes (Fig 4). For these insect parasitic nematodes, an appreciable probability of host mortality (>75%) is achieved at somewhere between 50-100+ infecting nematodes after two days. Importantly, it is worth noting that the insect species being infected—larvae of the greater wax moth (Galleria mellonella)—is known for and used in entomopathogenic nematode rearing because of its relatively weak and maladapted immune system [21]. In other, adapted, hosts, high probability of insect host mortality would likely necessitate an even greater number of parasites entering the host to facilitate successful infection.

Because successful infection is the primary objective of parasitic nematodes upon locating a host, it is interesting that environmental cues and learned experience can augment infection potential. Presence of and past exposure to plant volatiles can increase entomopathogenic nematode virulence. Enhanced virulence is plastic and can be learned based on past experience.

This plasticity can influence the lifecycle of nematodes in the natural environment and be appropriated for biological control of insect pests. Entomopathogenic nematodes emerging from their host cadaver likely are exposed to plant volatiles that may influence infection potential in similar situations in the future. Additionally, by isolating infection behavior in assays obviating long-distance recruitment, our results inform previous observations that recruitment alone is not sufficient to account for increased host insect mortality in comparatively large olfactometers [16]. This pattern of enhanced infection following volatile exposure can also inform biological control solutions where entomopathogenic nematodes can be exposed to volatile cues in-vitro in order to enhance their infection in agricultural fields.

The ability of nematode parasites to demonstrate infection plasticity based on environmental cues and past experience is not limited to management of entomopathogenic nematodes, however. Many nematode parasites of vertebrates, plants and insects demonstrate similar lifecycles and share common adaptations. Animal and plant nematode parasites, for example, respond strongly to host associated plant volatiles [22] and many share conserved pheromone signaling mechanisms [23]. Management and knowledge of environmental cues and past experience will likely aid in managing nematode parasites in a variety of settings: enhancing biological control with entomopathogenic nematodes and reducing infection of vertebrate and plant parasitic nematodes are current examples of potential practical application.
Materials and methods

Organisms
Entomopathogenic nematode infective juveniles of *Heterorhabditis indica* and *Steinernema diaprepes* were originally isolated from Florida citrus groves (near Homestead and Bartow Florida respectively) then cultivated in *Galleria mellonella* waxworm larvae until use in experiments [24]. This study was not a field trial and only involved laboratory work. Strains used in this study were originally isolated from natural populations in Florida at locations used by the University of Florida. Permissions for nematode collection are not required at those locations. The strains isolated from those locations and used in this study are standard laboratory strains used in a multitude of laboratory trials and similarly used in many of the references cited in this manuscript. Upon emerging from larvae, infective juveniles were collected in White traps then used within a week in bioassays [25].

Bioassays
Bioassay arenas consisted of one gram of clean autoclaved sand adjusted to 10% moisture by volume placed into each well of a 24 well plate (1.56cm bottom diameter). One host *G. mellonella* larva was added to each well along with respective volatile treatment. Following arena setup and application of volatile treatment, 1000 entomopathogenic nematode infective juveniles were added to each well. Either 24 or 48 hours later, mortality of *G. mellonella* was determined by observing responses to prodding with a needle. Following mortality determination, larvae were frozen. After freezing, larvae underwent pepsin digestion and infective juveniles that had entered the larvae were extracted and counted as previously described [26, 27].

Experimental design
To determine how the presence of plant volatiles influences entomopathogenic nematode infection, sand arenas with *G. mellonella* larvae were inoculated with 10 μl of pentane containing either 10 ng of pregeijerene (extracted from the roots of the Common Rue plant *Ruta graveolens*), 1 ng pregeijerene, 10 ng α-pinene (Sigma Aldrich, 98%), or 1 ng α-pinene. Twelve replications of each treatment were conducted.

To determine how prior exposure to plant volatiles affects entomopathogenic nematode infectivity, cohorts of 1000 infective juveniles were placed in vials containing 10ml DI water and 5 μl of either 10 ng/μl pregeijerene or 10 ng/μl α-pinene in pentane. Vials containing these exposed cohorts were incubated at 25˚C for 48 hours before washing the infective juveniles three times. For comparison, non-exposed cohorts were also established as above with the exception that 5 μl pentane was used in place of volatile treatment. These cohorts were then placed into arenas constructed as above and treated with either 10 ng of pregeijerene, 1 ng pregeijerene, 10 ng α-pinene, 1 ng α-pinene, or pentane such that volatile treatments matched pre-exposure. As above, twelve replications of each treatment were conducted.

Additionally, each of the trials above had three different controls each consisting of twelve replications. The first control consisted of host insects prepared as above to which no nematodes were added. This control was included to check for possible contamination by rogue entomopathogenic nematodes that could have (however unlikely) escaped culture. In all cases, these controls returned negative results; no nematodes were ever recovered and no host insects suffered mortality. The second control consisted of nematodes only where the host insect was prepared as above with the exception that no plant volatiles were included in the arena. This control was designed to establish a baseline level of infection in the absence of host environmental cues. The third control consisted of host insects treated with pentane only. This control
was designed to verify that the small amount of pentane solvent was not altering entomopathogenic nematode response. Because of the results of these controls, it is highly unlikely that pentane increases entomopathogenic nematode response. Pentane only controls resulted in either slightly less infection than \((P < 0.0001)\) or were not significantly different from no pentane controls \((P = 0.08)\).

**Analysis**

Entomopathogenic nematode infectivity was evaluated with Poisson regression. Species, treatment, hours post infection, replication number, exposure status and two factor interactions were considered for their ability to explain observed numbers of infective juveniles in the insect cadaver. Models were selected based on analysis of deviance, information criteria, goodness of fit metrics, psuedo \(R^2\) values, and residual diagnostics.

The effects and interactions between number of infective juveniles entering a cadaver, hours post infection, treatment, and nematode species on \(G.\ mellonella\) mortality was evaluated using logistic regression. Best fit models were chosen based on analysis of deviance, information criteria, goodness of fit metrics, psuedo \(R^2\) values, and residual diagnostics.

Data were collated in Microsoft Excel then imported into R version 3.3.1 [28] for analysis in the RStudio version 1.0.136 development environment [29]. The following packages facilitated analysis: *readxl* for the R-Excel interface [30], *tidyverse* for creating a tidy data environment [31], *car* for analysis of deviance [32], and *lsmeans* for post-hoc comparisons [33].

**Supporting information**

**S1 Fig. Baseline infection with volatile treatments.** Points and error bars denote mean number of nematodes recovered from the host insect cadaver and 95% confidence intervals respectively. Controls are nematode only controls; host insects were not treated with volatiles. (TIFF)

**S2 Fig. Infection following prior exposure to volatile treatments.** Points and error bars denote mean number of nematodes recovered from the host insect cadaver and 95% confidence intervals respectively. Controls are nematode only controls; host insects were not treated with volatiles. Exposed cohorts had experience with pregeijerene. (TIFF)

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References

1. Poulin R. Evolutionary ecology of parasites. Princeton university press; 2011.
2. Woolhouse ME, Webster JP, Domingo E, Charlesworth B, Levin BR. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature genetics. 2002; 32(4):569–577. https://doi.org/10.1038/ng1202-569 PMID: 12457190
3. Kennedy MW, Harnett W. Parasitic nematodes: molecular biology, biochemistry and immunology. CABI; 2013.
4. Moncada DM, Kammanadinti SJ, Chadee K. Mucin and Toll-like receptors in host defense against intestinal parasites. Trends in parasitology. 2003; 19(7):305–311. https://doi.org/10.1016/S1471-4922(03)00122-3 PMID: 12855381
5. Finkelman FD, Shea-Donohue T, Morris SC, Gildea L, Strait R, Madden KB, et al. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. Immunological reviews. 2004; 201(1):139–155. https://doi.org/10.1111/j.0105-2896.2004.00192.x PMID: 15361238
6. Grecnis RK. Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. Annual review of immunology. 2015; 33:201–225. https://doi.org/10.1146/annurev-immunol-032713-120218 PMID: 25533702
7. Jones JD, Dangl JL. The plant immune system. Nature. 2006; 444(7117):323–329. https://doi.org/10.1038/nature05286 PMID: 17108957
8. Lin B, Zhuo K, Chen S, Hu L, Sun L, Wang X, et al. A novel nematode effector suppresses plant immunity by activating host reactive oxygen species-scavenging system. New Phytologist. 2016; 209(3):1159–1173. https://doi.org/10.1111/nph.13701 PMID: 26484653
9. Rahatkah Z, Karimi J, Ghadamyari M, Brivio MF. Immune defenses of Agriotes lineatus larvae against entomopathogenic nematodes. BioControl. 2015; 60(5):641–653. https://doi.org/10.1007/s10526-015-9678-z
10. Rifkin M, Seow HF, Jackson D, Brown L, Wood P. Defence against the immune barrage: Helminth survival strategies. Immunology & Cell Biology. 1996; 74(6).
11. Morrill A, Forbes MR. Aggregation of Infective Stages of Parasites as an Adaptation and Its Implications for the Study of Parasite-Host Interactions. The American Naturalist. 2016; 187(2):225–235. https://doi.org/10.1086/684508 PMID: 26807749
12. Wang Y, Gaugler R, Cui L. Variations in immune response of Popillia japonica and Acheta domesticus to Heterorhabditis bacteriophora and Steinernema species. Journal of Nematology. 1994; 26(1):11. PMID: 19279963
13. Westerman P. Aggregation of Entomopathogenic Nematodes, Heterorhabditis spp. andSteinernemasmpp., among Host Insects at 9 and 20 °C and Effects on Efficacy. Journal of invertebrate pathology. 1999; 73(2):206–213. https://doi.org/10.1006/jipa.1998.4801 PMID: 10066401
14. Fushing H, Zhuo L, Shapiro-Ilan DJ, Campbell JF, Lewis EE. State-space based mass event-history model i: many decision-making agents with one target. The annals of applied statistics. 2008; 2(4):1503. https://doi.org/10.1214/08-AOAS189 PMID: 19421335
15. Willett DS, Alborn HT, Duncan LW, Stelinski LL. Social networks of educated nematodes. Scientific reports. 2015; 5. https://doi.org/10.1038/srep14388 PMID: 26404058
16. Willett DS, Alborn HT, Stelinski LL. Multitrophic Effects of Belowground Parasitoid Learning. Scientific Reports. 2017; 7. https://doi.org/10.1038/s41598-017-02193-2
17. Turlings TC, Hiltpold I, Rasmann S. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. Plant and Soil. 2012; 358(1-2):51–60. https://doi.org/10.1007/s11104-012-1295-3
18. Ali JG, Alborn HT, Stelinski LL. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by Diaprepes abbreviatus recruit entomopathogenic nematodes. Journal of chemical ecology. 2010; 36(4):361–368. https://doi.org/10.1007/s10886-010-9773-7 PMID: 20309617
19. Ali JG, Alborn HT, Stelinski LL. Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. Journal of Ecology. 2011; 99(1):26–35. https://doi.org/10.1111/j.1365-2745.2010.01758.x
20. Rasman S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, et al. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature. 2005; 434(7034):732–737. https://doi.org/10.1038/nature03451 PMID: 15815622
21. Li XY, Cowles R, Cowles E, Gaugler R, Cox-Foster D. Relationship between the successful infection by entomopathogenic nematodes and the host immune response. International journal for parasitology. 2007; 37(3):365–374. https://doi.org/10.1016/j.ijpara.2006.08.009 PMID: 17275827
22. Castelletto ML, Gang SS, Okubo RP, Tselikova AA, Nolan TJ, Platzer EG, et al. Diverse host-seeking behaviors of skin-penetrating nematodes. PLoS pathogens. 2014; 10(8):e1004305. https://doi.org/10.1371/journal.ppat.1004305 PMID: 25121736
23. Choe A, von Reuss SH, Kogan D, Gasser RB, Platzer EG, Schroeder FC, et al. Ascaroside signaling is widely conserved among nematodes. Current Biology. 2012; 22(9):772–780. https://doi.org/10.1016/j.cub.2012.03.024 PMID: 22503501
24. Kaya HK, Stock S. Techniques in insect nematology. Manual of techniques in insect pathology. 1997; 1:281–324. https://doi.org/10.1016/B978-012432555-5/50016-6
25. White G. A method for obtaining infective nematode larvae from cultures. Science. 1927; 66 (1709). https://doi.org/10.1126/science.66.1709.302-a PMID: 17749713
26. Shapiro DI, Lewis EE. Comparison of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. Environmental Entomology. 1999; 28(5):907–911. https://doi.org/10.1093/ee/28.5.907
27. Mauleon H, Briand S, Laumond C, Bonifassi E. Utilisation d’enzymes digestives pour l’étude du parasitisme des Steinernema et des Heterorhabditis envers les larves d’insectes. Fundamental and Applied Nematology. 1993; 16(2):185–186.
28. R Core Team. R: A Language and Environment for Statistical Computing; 2015. Available from: https://www.R-project.org/.
29. RStudio Team. RStudio: Integrated Development Environment for R; 2015. Available from: http://www.rstudio.com/.
30. Wickham H. readxl: Read Excel Files; 2016. Available from: https://CRAN.R-project.org/package=readxl.
31. Wickham H. tidyverse: Easily Install and Load‘Tidyverse’ Packages; 2016. Available from: https://CRAN.R-project.org/package=tidyverse.
32. Fox J, Weisberg S. An R Companion to Applied Regression. 2nd ed. Thousand Oaks CA: Sage; 2011. Available from: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.
33. Lenth RV. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software. 2016; 69(1):1–33. https://doi.org/10.18637/jss.v069.i01