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ALTERNATIVE PATHS TO SUCCESS IN A PARASITE COMMUNITY: WITHIN-HOST COMPETITION CAN FAVOR HIGHER VIRULENCE OR DIRECT INTERFERENCE

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Selection imposed by coinfection may vary with the mechanism of within-host competition between parasites. Exploitative competition is predicted to favor more virulent parasites, whereas interference competition may result in lower virulence. Here, we examine whether exploitative or interference competition determines the outcome of competition between two nematode species (*Steinernema* spp.), which in combination with their bacterial symbionts (*Xenorhabdus* spp.), infect and kill insect hosts. Multiple isolates of each nematode species, carrying their naturally associated bacteria, were characterized by (1) the rate at which they killed insect hosts, and by (2) the ability of their bacteria to interfere with each other's growth via bacteriocidal toxins called "bacteriocins." We found that both exploitative and interference abilities were important in predicting which species had a selective advantage in pairwise competition experiments. When nematodes carried bacteria that did not interact via bacteriocins, the faster killing isolate had a competitive advantage. Alternatively, nematodes could gain a competitive advantage when they carried bacteria able to inhibit the bacteria of their competitor. Thus, the combination of nematode/bacterial traits that led to competitive success depended on which isolates were paired, suggesting that variation in competitive interactions may be important for maintaining species diversity in this community.

KEY WORDS: **Allelopathy, bacteriocins, coexistence, coinfection, diversity, entomopathogenic nematodes, exploitative competition, interspecific competition, *Steinernema*, *Xenorhabdus*.**

Competition is a major force influencing ecological communities and phenotypic evolution. Exploitation competition, whereby competing individuals use the same resource, can select for faster growing or more efficient competitors (e.g., Mueller 1988; terHorst 2011). It can also lead to the evolution of interference competition (Roughgarden 1983), whereby individuals prevent each other from using resources either by chemical means or direct contact (Schoener 1983). For parasites, competition may occur within the host (Smith and Holt 1996), and parasites have been shown to shift their use of host tissues in response to interspecific competition (Stock and Holmes 1988; Friggens and Brown 2005). In addition, numerical responses to interspecific competition can be severe enough to result in competitive exclusion of one species from the co-infected host (Dobson 1985; Kuris and Lafferty 1994).

The role of within-host competition on parasite traits has been especially influential in models of the evolution of virulence. These models view virulence as the result of parasite growth and

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host-resource consumption. Traditionally, most models assumed that parasites competed within the host via exploitation competition. Thus, within-host selection would favor faster growing and more virulent parasites (Levin and Pimentel 1981; Bremermann and Pickering 1983). However, when parasites compete within the host via interference competition, selection may favor increased investment in interference-related traits, such as anticompetitor toxins. Because the production of these toxins is thought to be energetically costly to the producing species, and the toxins reduce the growth of the competitor species, within-host selection via interference competition is thought to slow the growth of the total parasite population within the host and reduce virulence in co-infected hosts (Gardner et al. 2004; Massey et al. 2004; Bashey et al. 2012). Given that these different mechanisms of within-host interactions may change the evolution of parasites and their virulent effects on hosts (see Choisy and de Roode 2010; Alizon and Lion 2011 for nuanced discussions of the feedbacks involved), it is important to better understand what parasite traits increase within-host competitive success.

Although exploitation competition is implicit in models of interference competition, the combined action of the two mechanisms on the evolution of parasite traits has not been explicitly modeled (Buckling and Brockhurst 2008). A key component of any such theory would be the rules for determining the outcome of within-host selection. For example, does dominance in an interference interaction necessitate within-host success? Or, could a faster growing species escape interference competition within a host? Furthermore, within-species variation in exploitative abilities or in investment in interference mechanisms may shift the outcome of competition between two competing species (Park et al. 1964). If these abilities trade-off, or if resistance to the negative impacts of competition evolves, then competitive interactions themselves may maintain variation within each species and facilitate species coexistence (Joshi and Thompson 1995; Lankau and Strauss 2007; Lankau 2008).

Here, we examine the potential for both exploitative and interference competition to affect the outcome of competition between sympatric isolates of two nematode species in the genus *Steinernema* (*Steinernema* sp. C3 and *S.* sp. C4, Bashey et al. 2011). Each of these soil-dwelling nematode species is involved in a mutualistic symbiosis with a specific species of bacteria in the genus *Xenorhabdus* (*X. bovienii* and *X. koppenhoeferi*, respectively). Together, the nematodes and bacteria are able to infect and kill a broad range of insect hosts (Peters 1996). Previously, we isolated nematodes and their bacteria from multiple soil samples taken from a single hillside (Hawlena et al. 2010a,b). From these soil samples, several distinct genotypes of the two *Xenorhabdus* species were identified. For each bacterial genotype, we characterized the potential for interference competition via the production of and sensitivity to bacteriocins, which are toxins produced by bacteria that are known for their ability to kill closely related bacteria (Riley and Gordon 1999; Riley and Chavan 2007). Moreover, there is evidence that bacteriocins produced by *Xenorhabdus* may affect the outcome of competition between nematode species (Sicard et al. 2005; Morales-Soto and Forst 2011). Based on these results, we predicted the outcome of mixed nematode species infections due to interference competition. Our predictions were based on the hypothesis that the ability of a bacterial symbiont to produce a bacteriocin capable of inhibiting the growth of a competing symbiont should confer a competitive advantage to its associated nematode.

We tested these predictions based on interference competition alongside predictions based on exploitative competition. Specifically, we hypothesized that the ability of a nematode isolate to grow quickly within the insect host is also crucial for competitive success. In the early stages of infection, nematodes must switch from a developmentally dormant, free-living state to an actively feeding and growing state. As nematodes begin to feed, they release their symbiotic bacteria, which reproduce independently of the nematode inside the insect (Sicard et al. 2004a). Several nematode and bacterial traits, as well as interactions between the two, have been implicated in explaining variation in virulence and parasitic success (e.g., Simoes 2000; Flores-Lara et al. 2007; Herbert Tran and Goodrich-Blair 2009). This initial stage of infection culminates in the death of the insect, and prior work with these isolates suggests that the speed at which nematodes are able to establish an infection and kill the host may be important for competitive dominance (Bashey et al. 2011). Thus, we used variation in the timing of host death in single-isolate infections, where nematodes carrying their naturally associated bacteria were exposed to an insect host, as a proxy of the exploitative ability of each isolate. We predicted that faster killing isolates should be competitively dominant. By testing hypotheses based on both mechanisms of competition in sympatric isolates, our study seeks to evaluate the relative importance of these mechanisms and the impact they may have on parasite evolution and community diversity.

Materials and Methods

STUDY SYSTEM

Nematodes in the genus *Steinernema* are free-living and nonfeeding in the soil as juveniles, but they require an insect host for development and reproduction. Each juvenile nematode carries symbiotic bacteria (genus: *Xenorhabdus*) in a specialized region of its intestine (Poinar 1966; Martens and Goodrich-Blair 2005). The symbiosis between the nematode and the bacteria can be viewed as a mutualism, with each partner contributing to the success of the symbiotic pair. From the bacteria's perspective, the

nematode provides transmission between insect hosts, as free-living *Xenorhabdus* have not been found in the soil and their ability to survive in the soil is low (Morgan et al. 1997). Nematodes can persist in the soil for months, and may travel up to a few meters in search of new insect hosts (Schroeder and Beavers 1987; Strong 2002). The nematode is also critical for penetrating the insect cuticle and it forms the first line of resistance/attack on the insect immune system. The nematode cuticle and surface coat proteins can prevent the insect encapsulation response (Dunphy and Webster 1987; Wang and Gaugler 1999; Brivio et al. 2002). Further, the nematode releases several products that can disable the insect immune system (Goetz et al. 1981; Simoes 2000) and cause insect death (Burman 1982) when the bacteria are experimentally removed. These initial effects of the nematode are important to ensure bacterial release and may additionally create a more favorable environment for bacterial survival and growth (Wang and Gaugler 1999).

The nematodes actively release their bacteria (Snyder et al. 2007) into the insect hemocoel, where the bacteria grow independently of the nematodes (Sicard et al. 2004a). When injected into insects without the nematode, the bacteria are highly virulent, attacking the insect immune system and degrading insect tissues (see Herbert and Goodrich-Blair 2007; Richards and Goodrich-Blair 2009 for reviews of the bacterial factors involved in these processes). After insect death, the bacteria play a critical role in supporting nematode reproduction, which is greatly reduced or even absent without the bacteria (Sicard et al. 2003). Moreover, the association between the nematode and the bacteria is quite specific, with nematodes showing reduced reproductive success with nonnative bacteria (Sicard et al. 2004b; Chapuis et al. 2009). The nematode and bacteria re-associate as the insect carcass becomes depleted and the pair emerge into the soil (Popiel 1989; Martens et al. 2003). *Steinernema* nematodes are able to infect a wide range of insect hosts (Peters 1996); in this study, we use the greater wax moth *Galleria mellonella* as the insect host.

NEMATODE AND BACTERIAL ISOLATES

Nematodes were isolated from soil samples collected on a hillside at the Indiana University Research and Teaching Preserve, Moore's Creek, Monroe County, Indiana as described in Hawlena et al. (2010a). Briefly, soil samples were baited with larvae of *G. mellonella*, and each isolate was derived from nematodes that had emerged from a single caterpillar. Nematode isolates were maintained in dH20 at 8°C and cycled separately through *G. mellonella* for two additional passages in the laboratory before the experiment to minimize environmental differences across the isolates.

Based on sequencing of the 28S rRNA gene (Stock et al. 2001), nematode isolates were found to belong to two currently undescribed species in genus *Steinernema* (Bashey et al. 2011): one (*S.* sp. C3) in Clade 3 and one (*S.* sp. C4) in Clade 4 (Uribe-Lorio et al. 2007). The bacteria species associated with each isolate was identified by sequencing of the 16S rRNA gene (Tailliez et al. 2006; Hawlena et al. 2010a). *S.* sp. C3 nematodes were associated with *X. bovienii*, whereas *S.* sp. C4 nematodes were associated with *X. koppenhoeferi*. The bacteria were further characterized based on enterobacterial repetitive intergenic consensus sequences and BOX-element genomic fingerprints as described in Hawlena et al. (2010a,b). Four distinct genotypes of *X. bovienii* (hereafter, B1–B4) and three distinct genotypes of *X. koppenhoeferi* (hereafter, K1–K3) were characterized.

The potential for interference competition interactions among the bacterial symbionts was characterized by an in vitro assay, whereby each genotype was grown clonally and induced with mitomycin C (Hawlena et al. 2012). Cell-free supernatants from the induced cultures (actor) were then tested against potential sensitive cultures (recipient) by placing a drop of the actor supernatant on soft agar seeded with the recipient colony and then scoring for growth inhibition. Each genotype was subject to at least two separate inductions and each actor/recipient pair was tested on at least two separate occasions. To ensure the absence of false-positive results, negative control tests were also conducted whereby we tested each isolate's response as a recipient by applying a supernatant that was produced at the same time and according to the same protocol, but without the addition of bacteria. None of these negative controls showed inhibition. All genotypes tested, except K1 and B1, were found to produce bacteriocins (Hawlena et al. 2010a). Moreover, we found no difference in the magnitude of the inhibition resulting from the bacteriocins produced by the two species (data not shown).

EXPERIMENTAL DESIGN

We predicted the outcome of interference competition between the two nematode species based on the bacteriocin genotype associated with each nematode isolate (Fig. 1). As subspecific variation in each isolate has only been characterized due to the bacterial genotypes, we will refer to our nematode isolates based on their bacterial genotypes. All infections done in this study involve the nematodes carrying their symbiotically associated bacteria. For brevity, we will refer to *S.* sp. C3—*X. bovienii* isolates as "BOV" and *S.* sp. C4—*X. koppenhoefferi* isolates as "KOP." To assess differences in exploitative competition between the nematode isolates, we performed single-isolate infections of all seven genotypes to determine the speed of the initial phase of infection and differences in the timing of host death. To determine the outcome of competition, 12 types of mixed-species infections were done (all combinations in Fig. 1). Two replicate sets of infections were done, using different nematode isolates of each genotype, except for B1 and B3, which were only characterized from one nematode isolate each. For each of the infection treatments ($n = 38$),

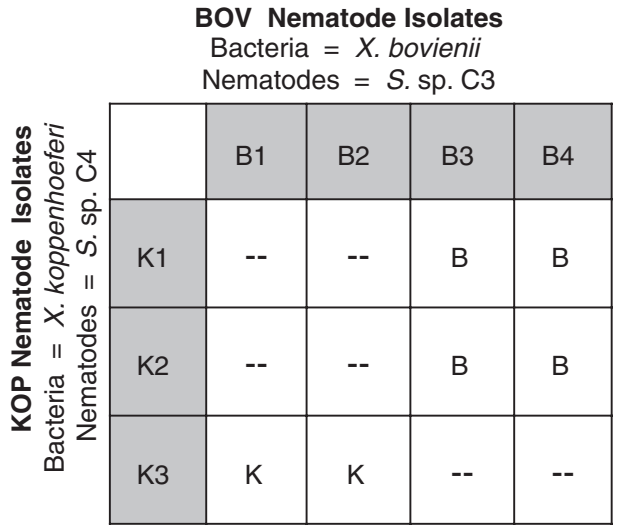

Figure 1. Predicted outcome of the 12 mixed nematode species infections based on bacteriocin phenotypes of their bacterial symbionts. Three KOP and four BOV bacterial genotypes were identified. "B" indicates BOV is the predicted winner and "K" indicates KOP is the predicted winner, as in each case, one bacterial genotype was able to produce a bacteriocin that inhibited the growth of the other genotype in vitro. In cases where there was no bacteriocin-based interaction between the two bacterial genotypes, "–" indicates that no prediction based on interference competition was made a priori. Twenty caterpillars were infected with each of the seven genotypes in single nematode species exposures, and with each of 12 mixed nematode species pairings. Two replicate sets of infections were done, using different nematode isolates where possible.

20 caterpillars were individually exposed to 50 nematodes, either all of one species, or a 50:50 mixture of two species as described previously (Bashey et al. 2011).

Infected hosts were kept at 18°C and examined for mortality up to five times per day for five days post infection. Caterpillars were scored as dead, if they did not move in response to being touched by a probe. Seven days post infection, caterpillars were transferred to White traps (White 1927; Bashey et al. 2007) for maintenance of the infection and collection of emerging nematodes. Nematodes were allowed to emerge from the host cadaver for 50 days post infection at which time the caterpillars were discarded. Nematodes were stored in dH20 at 8°C until analyzed for nematode and bacteria species identification. The nematode species emerging from each mixed infection was identified based on differences in nematode behavior, as BOV exhibit a tight curling behavior when kept cold, whereas KOP does not. Identifications were verified in a subset of samples by PCR-RFLP of the nematode 28S rRNA gene and by bacterial isolations, which can detect a species mix as low as 10% or 1% (respectively), as described in Bashey et al. (2011). Nematodes and bacteria resulting from each insect host were categorized as belonging to one symbiotic pair or the other. Nematodes emerging from a total of 252 insect hosts were examined across the 24 mixed infections. Only one host, which had very low number of emerging nematodes, was found to have a mixture of both nematode species, and was excluded from the analysis.

STATISTICAL ANALYSES

To determine differences in insect mortality rate among infection treatments, we performed Cox (proportional hazards) regressions using the Phreg Procedure in SAS. Estimates of the median time of host death (LT50) were determined by the Lifetest Procedure. We performed a logistic regression using the Glimmix Procedure to evaluate the effect of bacteriocin phenotype and mortality-rate differences on the outcome of competition (i.e., which species, KOP or BOV, emerged from each host). Genotypes were considered as random effects. The mortality-rate difference was calculated as the difference in the hazard ratio of the two isolates based on a proportional hazards regression of the single isolate infections.

Results

Overall, there was no difference in the mortality rate of insect hosts infected with either of the two nematode species ($\chi^2 = 0.1619$, df $= 1$, $P = 0.6874$). However, within each nematode species, isolates carrying the different bacterial genotypes varied considerably from each other in how fast they induced host death (BOV: $\chi^2 = 68.08$, df $= 3$, $P < 0.0001$; KOP: $\chi^2 = 22.82$, df $= 2$, $P < 0.0001$; Fig. 2). Replicate infections of nematode isolates carrying the same bacterial genotype did not vary significantly from each other ($P > 0.15$, Fig. 2), except for the two isolates of K1 ($\chi^2 = 15.05$, df $= 1$, $P = 0.0001$). These two isolates of K1 resulted from two soil samples collected over 60 m apart. In contrast, the other replicate infections resulted from the same nematode isolates or from isolates that originated from caterpillars exposed to soil sample collected less than 2 m apart. Thus, there could be more genetic variation between the two K1 replicates than between the other replicates; although, we have no additional nematode or bacterial markers to distinguish them.

Variation in the competitive outcome was explained by mortality-rate differences as estimated from single-isolate infections ($F_{1,14} = 16.98$, $P = 0.0010$), superiority in interference competition as based on the bacteriocin phenotypes ($F_{2,14} = 3.97$, $P = 0.0431$), and the interaction of these two factors ($F_{2,14} = 5.09$, $P = 0.0218$). In the pairings where there was no bacteriocin-based interference between the bacterial symbionts (open circles and dashed lines in Fig. 3), the species that induced faster host death when inoculated singly was dominant in competition ($F_{1,6} = 10.32$, $P = 0.0183$). Thus, in some pairings, the BOV isolates killed the insect hosts faster and emerged from the majority

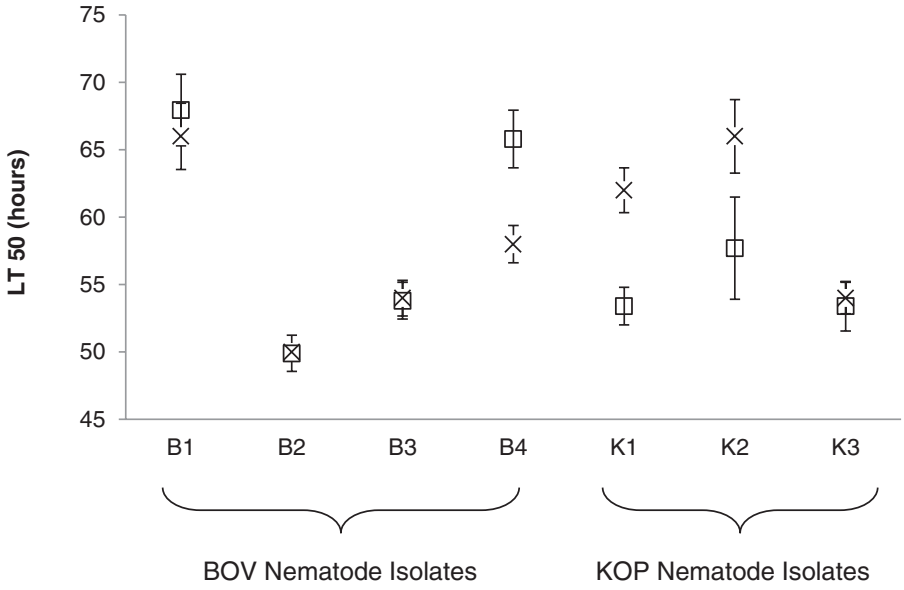

Figure 2. Median (±1 SE) time to death for caterpillars infected with each nematode isolate (carrying its naturally associated bacterial symbionts). Isolates are characterized by bacterial genotypes; although, nematode traits may vary as well. X's and squares indicate replicate infections of each bacterial genotype.

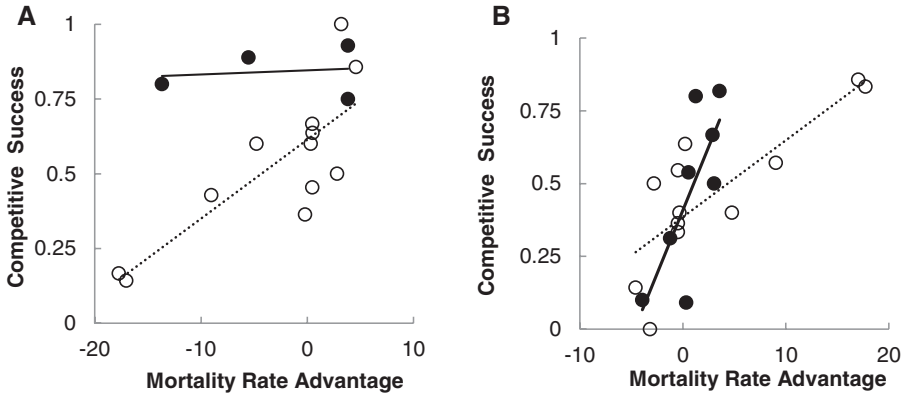

Figure 3. Competitive success of (A) KOP and (B) BOV nematodes in mixed nematode species infections as a function the mortality-rate advantage of each species and whether that species was the predicted winner based on bacteriocin assays (filled circles and solid lines) or whether there was no bacteriocin interaction between the bacterial symbionts (open circles and dashed lines). The competitive success represents the proportion of caterpillar hosts from which each species emerged. Values above 0.5 represent competitive advantage for the target species (KOP in A and BOV in B). The mortality-rate advantage was calculated as the difference in the hazard ratio of the two isolates based on the proportional hazards regression of each isolate alone. Positive values of the mortality-rate difference indicate that the target species induced a faster host death than the other. Note the open circles in each panel show the same data from the perspective of each species.

of hosts, whereas in other pairings, KOP killed faster and emerged from the majority of hosts. Additionally, in pairings where the rate of host killing did not differ between the species (mortality rate advantage ~0), an approximately equal number of hosts gave rise to nematodes of each species (mean competitive success ~0.5).

For the pairings where KOP was predicted to win based on the bacteriocin phenotypes (closed circles and solid line in Fig. 3A), KOP nematodes emerged from significantly more hosts than in pairings where KOP was not the predicted winner ($F_{1,17} = 5.91$, $P = 0.0264$). Moreover, differences in insect mortality rate did not affect the competitive outcome (Fig. 3A). Thus, when KOP produced an inhibiting bacteriocin, it was competitively dominant over BOV competitors that were significantly faster at killing insect hosts.

Finally, in the pairings where BOV was predicted to win based on the bacteriocin phenotypes (closed circles and solid line in Fig. 3B), the effect of producing an inhibiting bacteriocin was minimal. Despite the ability of these BOV bacteria to

inhibit the growth of their KOP bacterial partner, BOV was only competitively dominant in cases where it also induced faster host death. The slope of the line describing the relationship between competitive success and the mortality-rate advantage is significantly steeper when BOV was predicted to win than when there was no bacteriocin-based interference ($F_{1,12} = 6.00$, $P = 0.0307$). This resulted in a significant competitive advantage to BOV for producing an inhibiting bacteriocin only when the mortality-rate advantage was greater than 3, but no detectible effect for lower values of the mortality-rate advantage.

Discussion

Within-host selection is a major force influencing the evolution of parasite traits. Parasites may interact within a host via a variety of different mechanisms. For example, numerous models assume that faster growing, more virulent parasites will be favored by within-host selection (e.g., Levin and Pimentel 1981; Bremermann and Pickering 1983); while others predict interference competition could lead to the selection of less-virulent parasites (e.g., Gardner et al. 2004). Here, we examine multiple, sympatric isolates of two nematode species (carrying their naturally associated bacteria) and find that it is important to understand both the exploitative and interference abilities of a parasite to predict the nature of within-host selection. We found that different isolates of each species varied significantly in the speed at which they induced death of a caterpillar host (Fig. 2). We also found that, in the absence of interference activity, faster host killing was a significant predictor of competitive success in mixed-species infections (Fig. 3). However, we also found that interference competition via the production of bacteriocins can overcome a mortality-rate difference (Fig. 3A). Thus, in some cases, a slower killing parasite may have higher within-host fitness, if it is able to interfere with the growth of its competitor. These variable outcomes between sympatric isolates may prevent the dominance of a single genotype over others, as a fast killing genotype could be outcompeted by a bacteriocin-producer, but at the same time this producer could be outcompeted by another fast killing, nonsensitive genotype. Therefore, variation in competitive interactions due to exploitative and interference mechanisms may contribute to the maintenance of diversity within this community.

Our work supports the common assumption that faster host exploitation can result in within-host competitive success. We found that in the absence of bacteriocin-based inhibitions, faster killing isolates were competitively dominant over slower killing isolates (Fig. 3). Empirical support for a link between competitive success and faster host exploitation has been found in only a handful of systems (e.g., Ishii et al. 2002; de Roode et al. 2005; Staves and Knell 2010) and thus should be demonstrated rather than assumed. Additionally, even within a single system, parasite fitness can vary depending on the competitor. For example, in the parasitic fungus *Metarhizium anisopliae*, faster killing strains were competitively dominant in intraspecific competition, but were worse at interspecific competition presumably because they did not invest in costly mechanisms of interference competition (Staves and Knell 2010). We find similar results in that the traits that increase parasite fitness vary with the competitor and the mechanisms of competition. For example, when KOP parasites are faced with a nonsensitive BOV competitor, faster host killing is key to within-host competitive success; however, when encountering a sensitive competitor, faster killing is no longer important (Fig. 3A). These results suggest that selection on the host exploitation rate will vary not only with the frequency of coinfections, but with the identity of the competitors.

The two nematode species we examined differed in the importance of their symbionts' bacteriocins for competitive success. For KOP, production of an inhibiting bacteriocin was a strong predictor of success in within-host competition. In fact, even when a KOP isolate took several hours longer to kill insect hosts when singly infected than its BOV competitor, production of an inhibitory bacteriocin allowed KOP to emerge from significantly more hosts (Fig. 3A). In contrast, bacteriocin production by BOV resulted only in the minor effect of augmenting the competitive advantage gained by faster host killing (Fig. 3B). Thus, when BOV was at a mortality-rate disadvantage, bacteriocin production resulted in no increase in competitive success. The reason for this difference between species is not known. One explanation could be a difference between bacteriocin production between the in vitro assay and the in vivo competitions. Work on antibiotic production in the closely related bacterium *Photorhabdus luminescens* demonstrates that in vivo production levels can be much lower than that obtained from liquid cultures (Hu et al. 1999). Alternatively, differences between the bacterial symbionts in their within-host growth rates may lead to asymmetric effects of bacteriocin production. When inoculated asymbiotically into a caterpillar, *X. koppenhoeferi* grows significantly faster than *X. bovienii* (unpublished data and Bashey et al. 2012). This may prevent *X. bovienii* from mounting an effective attack on *X. koppenhoeferi*, unless *X. bovienii* has slight head start afforded by its nematode. Finally, these differences may also represent different ecological roles of bacteriocin production in the two species, where perhaps a major benefit of bacteriocin in *X. bovienii* comes from intraspecific interactions or to prevent invasion of an already infected host.

In conclusion, our study highlights that both interference competition and exploitation competition are important to parasite fitness. Uniquely, our study finds this variation is occurring between different sympatric isolates of two parasites. That such diversity of interactions is found on a local scale suggests that

these interactions themselves may be important to the maintenance of diversity in this community.

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LITERATURE CITED

Alizon, S., and S. Lion. 2011. Within-host parasite cooperation and the evolution of virulence. Proc. R. Soc. Lond. B. Biol. Sci. 278:3738–3747.

Bashey, F., L. T. Morran, and C. M. Lively. 2007. Coinfection, kin selection, and the rate of host exploitation by a parasitic nematode. Evol. Ecol. Res. 9:947–958.

Bashey, F., C. Reynolds, T. Sarin, and S. K. Young. 2011. Virulence and competitive ability in an obligately killing parasite. Oikos 120:1539–1545.

Bashey, F., S. K. Young, H. Hawlena, and C. M. Lively. 2012. Spiteful interactions between sympatric natural isolates of *Xenorhabdus bovienii* benefit kin and reduce virulence. J. Evol. Biol. 25:431–437.

Bremermann, H. J., and J. Pickering. 1983. A game-theoretical model of parasite virulence. J. Theor. Biol. 100:411–426.

Brivio, M. F., M. Pagani, and S. Restelli. 2002. Immune suppression of *Galleria mellonella* (Insecta, Lepidoptera) humoral defenses induced by *Steinernema feltiae* (Nematoda, Rhabditida): involvement of the parasite cuticle. Exp. Parasitol. 101:149–156.

Buckling, A., and M. Brockhurst. 2008. Kin selection and the evolution of virulence. Heredity 100:484–488.

Burman, M. 1982. *Neoaplenctana carpocapsae*: toxin production by axenic insect parasitic nematodes. Nematologica 28:62–70.

Chapuis, É., V. Emelianoff, V. Paulmier, N. Le Brun, S. Pagès, M. Sicard, and J. B. Ferdy. 2009. Manifold aspects of specificity in a nematode–bacterium mutualism. J. Evol. Biol. 22:2104–2117.

Choisy, M., and J. C. de Roode. 2010. Mixed infections and the evolution of virulence: effects of resource competition, parasite plasticity, and impaired host immunity. Am. Nat. 175:E105–E118.

de Roode, J. C., R. Pansini, S. J. Cheesman, M. E. H. Helinski, S. Huijben, A. R. Wargo, A. S. Bell, B. H. K. Chan, D. Walliker, and A. F. Read. 2005. Virulence and competitive ability in genetically diverse malaria infections. Proc. Natl. Acad. Sci. USA 102:7624–7628.

Dobson, A. P. 1985. The population dynamics of competition between parasites. Parasitology 91:317–347.

Dunphy, G. B., and J. M. Webster. 1987. Partially characterized components of the epicuticle of dauer juvenile steinernema feltiae and their influence on hemocyte activity in *Galleria mellonella*. J. Parasitol. 73:584–588.

Flores-Lara, Y., D. Renneckar, S. Forst, H. Goodrich-Blair, and P. Stock. 2007. Influence of nematode age and culture conditions on morphological and physiological parameters in the bacterial vesicle of *Steinernema carpocapsae* (Nematoda : Steinernematidae). J. Invertebr. Pathol. 95:110–118.

Friggens, M. M., and J. H. Brown. 2005. Niche partitioning in the cestode communities of two elasmobranchs. Oikos 108:76–84.

Gardner, A., S. A. West, and A. Buckling. 2004. Bacteriocins, spite and virulence. Proc. R. Soc. Lond. B. Biol. Sci. 271:1529–1535.

Goetz, P., A. Boman, and H. G. Boman. 1981. Interactions between insect immunity and an insect-pathogenic nematode with symbiotic bacteria. Proc. R. Soc. Lond. B. Biol. Sci. 212:333–350.

Hawlena, H., F. Bashey, and C. M. Lively. 2010a. The evolution of spite: population structure and bacteriocin-mediated antagonism in two natural populations of *Xenorhabdus* bacteria. Evolution 64:3198–3204.

Hawlena, H., F. Bashey, H. Mendes Soares, and C. M. Lively. 2010b. Spiteful interactions in a natural population of the bacterium *Xenorhabdus bovienii*. Am. Nat. 175:374–381.

———. 2012. Bacteriocin-mediated interactions within and between coexisting species. Ecol. Evol. 2:2521–2526.

Herbert, E. E., and H. Goodrich-Blair. 2007. Friend and foe: the two faces of *Xenorhabdus nematophila*. Nat. Rev. Microbiol. 5:634–646.

Herbert Tran, E. E., and H. Goodrich-Blair. 2009. CpxRA contributes to *Xenorhabdus nematophila* virulence through regulation of lrhA and modulation of insect immunity. Appl. Environ. Microbiol. 75:3998–4006.

Hu, K., J. Li, and J. M. Webster. 1999. Nematicidal metabolites produced by *Photorhabdus luminescens* (Enterobacteriaceae), bacterial symbiont of entomopathogenic nematodes. Nematology 1:457–469.

Ishii, T., J. Takatsuka, M. Nakai, and Y. Kanimi. 2002. Growth characteristics and competitive abilities of a nucleopolyhedrovirus and an entomopoxvirus in larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae). Biol. Control 23:96–105.

Joshi, A., and J. N. Thompson. 1995. Alternative routes to the evolution of competitive ability in two competing species of *Drosophila*. Evolution 49:616–625.

Kuris, A. M., and K. D. Lafferty. 1994. Community structure: larval trematodes in snail hosts. Annu. Rev. Ecol. Syst. 25:189–217.

Lankau, R., and S. Strauss. 2007. Mutual feedbacks maintain both genetic and species diversity in a plant community. Science 317:1561–1563.

Lankau, R. 2008. A chemical trait creates a genetic trade-off between intra- and interspecific competitive ability. Ecology 89:1181–1187.

Levin, S., and D. Pimentel. 1981. Selection of intermediate rates of increase in parasite-host systems. Am. Nat. 117:308–315.

Martens, E. C., and H. Goodrich-Blair. 2005. The *Steinernema carpocapsae* intestinal vesicle contains a subcellular structure with which *Xenorhabdus nematophila* associates during colonization initiation. Cell. Microbiol. 7:1723–1735.

Martens, E. C., K. Heungens, and H. Goodrich-Blair. 2003. Early colonization events in the mutalistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus nematophila* bacteria. J. Bacteriol. 185:3147–3154.

Massey, R., A. Buckling, and R. ffrench-Constant. 2004. Interference competition and parasite virulence. Proc. R. Soc. Lond. B. Biol. Sci. 271:785–788.

Morales-Soto, N., and S. A. Forst. 2011. The xnp1 P2-like tail synthesis gene cluster encodes xenorhabdicin and is required for interspecies competition. J. Bacteriol. 193:3624–3632.

Morgan, J. A. W., V. Kuntzelmann, S. Tavernor, M. A. Ousley, and C. Winstanley. 1997. Survival of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* in water and soil. J. Appl. Microbiol. 83:665–670.

Mueller, L. D. 1988. Evolution of competitive ability in *Drosophila* by density-dependent natural selection. Proc. Natl. Acad. Sci. USA 85:4383–4386.

Park, T., P. H. Leslie, and D. B. Mertz. 1964. Genetic strains and competition in populations of *Tribolium*. Physiol. Zool. 37:97–162.

Peters, A. 1996. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. Biocontrol. Sci. Technol. 6:389–402.

Poinar, G. O. J. 1966. The presence of *Achromobacter nematophilus* in the infective stage of a *Neoplactana* sp. (Steinernema: Nematoda). Nematologica 12:105–108.

Popiel, I. 1989. Infective juvenile formation in the insect parasitic nematode *Steinernema feltiae*. Parasitology 99:77–81.

Richards, G. R., and H. Goodrich-Blair. 2009. Masters of conquest and pillage: *Xenorhabdus nematophila* global regulators control transitions from virulence to nutrient acquisition. Cell. Microbiol. 11:1025–1033.

Riley, M. A., and M. A. Chavan, eds. 2007. Bacteriocins: ecology and evolution. Springer-Verlag, Berlin.

Riley, M. A., and D. M. Gordon. 1999. The ecological role of bacteriocins in bacterial competition. Trends. Microbiol. 7:129–133.

Roughgarden, J. 1983. Competition and theory in community ecology. Am. Nat. 122:583–601.

Schoener, T. W. 1983. Field experiments on interspecific competition. Am. Nat. 122:240–285.

Schroeder, W. J., and J. B. Beavers. 1987. Movement of the entomogenous nematodes of the families *Heterorhabditidae* and *Steinernematidae* in soil. J. Nematol. 19:257–259.

Sicard, M., K. Brugirard-Ricaud, S. Pages, A. Lanois, N. E. Boemare, M. Brehelin, and A. Givaudan. 2004a. Stages of infection during the tripartite interaction between *Xenorhabdus nematophila*, its nematode vector, and insect hosts. Appl. Environ. Microbiol. 70:6473–6480.

Sicard, M., J. B. Ferdy, S. Pages, N. Le Brun, B. Godelle, N. Boemare, and C. Moulia. 2004b. When mutualists are pathogens: an experimental study of the symbioses between Steinernema (entomopathogenic nematodes) and Xenorhabdus (bacteria). J. Theor. Biol. 17:985–993.

Sicard, M., N. Le Brun, S. Pages, B. Godelle, N. Boemare, and C. Moulia. 2003. Effect of native *Xenorhabdus* on the fitness of their *Steinernema* hosts: contrasting types of interactions. Parasitol. Res. 91:520–524.

Sicard, M., J. Tabart, N. E. Boemare, O. Thaler, and C. Moulia. 2005. Effect of phenotypic variation in *Xenorhabdus nematophila* on its mutualistic relationship with the entomopathogenic nematode *Steinernema carpocapsae*. Parasitology 131:687–694.

Simoes, N. 2000. Pathogenicity caused by high virulent and low virulent strains of *Steinernema carpocapsae* to *Galleria mellonella*. J. Invertebr. Pathol. 75:47–54.

Smith, V. H., and R. D. Holt. 1996. Resource competition and within-host disease dynamics. Trends. Ecol. Evol. 11:386–389.

Snyder, H., S. P. Stock, S. Kim, Y. Flores-Lara, and S. Forst. 2007. New insights into the colonization and release process of *Xenorhabdus nematophila* and the morphology and ultrastructure of the bacterial receptacle of its nematode host *Steinernema carpocapsae*. Appl. Environ. Microbiol. 73:5338–5346.

Staves, P. A., and R. J. Knell. 2010. Virulence and competitiveness: testing the relationship during inter- and intraspecific mixed infections. Evolution 64:2643–2652.

Stock, S. P., J. F. Campbell, and S. Nadler. 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characteristics. J. Parasitol. 87:877–889.

Stock, T. M., and J. C. Holmes. 1988. Functional relationships and microhabitat distributions of enteric helminths of grebes (Podicipedidae): the evidence for interactive communities. J. Parasitol. 74:214–227.

Strong, D. 2002. Populations of entomopathogenic nematodes in foodwebs. Pp. 225–241 *in* R. Gaugler, ed. Entomopathogenic nematodes. CABI, New York.

Tailliez, P., S. Pages, N. Ginibre, and N. Boemare. 2006. New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species. Int. J. Syst. Evol. Microbiol. 56:2805–2818.

terHorst, C. P. 2011. Experimental evolution of protozoan traits in response to interspecific competition. J. Theor. Biol. 24:36–46.

Uribe-Lorio, L., M. Mora, and P. Stock. 2007. *Steinernema costaricense* n. sp. and *S. puntauvense* n. sp. (Rhabditida: Steinernematidae), two new entomopathogenic nematodes from Costa Rica. Syst. Parasitol. 68:167–182.

Wang, Y., and R. Gaugler. 1999. *Steinernema glaseri* surface coat protein suppresses the immune response of *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. Biol. Control 14:45–50.

White, G. F. 1927. A method for obtaining nematode larvae from cultures. Science 66:302–303.

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