Co-infection, kin selection, and the rate of host exploitation by a parasitic nematode

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ABSTRACT

Hypothesis: Under exploitative competition, unrelated parasites should reproduce at a faster rate than related parasites.

Organisms: The parasitic nematode *Steinernema carpocapsae* and the insect host *Galleria mellonella*.

Methods: We created ten replicate lines each of high-migration (unrelated) and low-migration (related) nematode populations, and allowed them to evolve for 20 host passages.

Results: We found no difference between treatments in the number of juvenile nematodes produced. However, juvenile nematodes began to emerge significantly sooner in the low-migration treatment, suggesting a faster rate of host exploitation. Host mortality rate was also higher in the low-migration lines.

Conclusion: The results were contrary to predictions based on exploitative competition among the nematodes.

Keywords: competition, evolution of virulence, experimental evolution, kin selection, *Steinernema carpocapsae, Xenorhabdus nematophila*.

INTRODUCTION

Parasites face an interesting conundrum. They exploit their hosts in order to grow and reproduce, but over-exploitation can reduce parasite fitness by reducing transmission success. The dominant theoretical framework regarding the evolution of virulence relies on this trade-off between host exploitation and transmission to predict the evolution of intermediate levels of host exploitation (Levin and Pimentel, 1981; Anderson and May, 1982; May and Anderson, 1983). However, when multiple unrelated parasites compete within a host, higher levels of host exploitation are predicted to evolve (Bremermann and Pickering, 1983; Frank, 1992; May and Nowak, 1995; van Baalen and Sabelis, 1995). This is because parasites that restrict their level of host exploitation can be out-competed within the host by faster growing parasites. This within-host selection becomes less important as within-host relatedness increases and, as a consequence, the rate of host exploitation reduces to the single-infection case if co-infecting parasites are highly related (Frank, 1996; Chao *et al.*, 2000; Lively, 2005).

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Correlative studies largely support this theory (Herre, 1993; Pickering *et al.*, 2000), but the results from experimental studies have been mixed. For example, virulence has been shown to increase when hosts are infected by two unrelated pathogen strains (Gingery and Nault, 1990; Taylor *et al.*, 1998; Davies *et al.*, 2002), but a positive correlation between virulence and parasite reproductive rate was found in only one of these cases (Davies *et al.*, 2002). Furthermore, more prudent parasite strategies have evolved in response to reduced migration (Kerr *et al.*, 2006; Boots and Mealor, 2007); however, in both studies, parasites showed restraint by infecting fewer hosts, rather than by exploiting a single host more prudently.

Here we present the results of an experimental study that examined the rate of host exploitation by the entomopathogenic nematode *Steinernema carpocapsae*. *Steinernema carpocapsae* and its symbiotic bacteria quickly kill the insect host, and then reproduce within the host carcass. Resource competition within the host carcass reduces the quality of the nematodes that emerge (Selvan *et al.*, 1993; Nguyen and Smart, 1995). These emerging nematodes are non-feeding, and their energy reserves are critical for survival and infection success (Qui and Bedding, 2000a, 2000b). Thus, because host death precedes parasite reproduction and greater host exploitation affects transmission success, this system provides an opportunity to study the effect of relatedness on host exploitation, while minimizing the confounding effect of host immune responses. We tested the hypothesis that manipulation of population structure would cause a change in host exploitation. Specifically, we anticipated that, as relatedness increased in response to restricted migration: (1) transmission-stage nematodes would emerge from the host at an earlier date in high-migration lines than in low-migration lines; (2) hosts infected with nematodes from the high-migration treatment would produce a greater number of transmission-stage nematodes once emergence began; and (3) the number of transmission-stage nematodes emerging from a given host would decline faster with time in the high-migration treatment, indicating a faster exhaustion of the host resources.

MATERIALS AND METHODS

Study system

Steinernema carpocapsae is an entomopathogenic nematode that infects a broad range of insects (Peters, 1996). Infective juvenile nematodes enter the host through natural openings and release their symbiotic bacteria, *Xenorhabdus nematophila*. As a result of the action of both the bacteria (Boemare and Akhurst, 1988; Dunphy and Webster, 1988) and the nematodes (Goetz *et al.*, 1981; Burman, 1982; Simoes, 2000), the host dies within a few days. Although the bacteria and the nematodes require one another to complete their life cycles (Han and Ehlers, 2000), they exist separately inside the host. The nematodes feed on host tissues and bacteria, reproducing inside the host for one or more generations before host resources become limiting. The nematodes then become colonized by the bacteria, cease feeding, and emerge as infective juveniles. Infective juveniles are free-living and non-feeding and can survive for several months without encountering a new host (Gaugler, 2002).

Experimental protocol

Steinernema carpocapsae were obtained from three commercial sources: Integrated Biocontrol Systems Inc. (Greendale, IN: 'Sal' strain), Mellinger's Inc. (N. Lima, OH: an

unidentified strain), and Biocontrol Network (Brentwood, TN: 'All' strain). Equal concentrations of infective juvenile nematodes from each source were mixed together and used to infect hosts. We used the greater wax moth caterpillar, *Galleria mellonella*, as the insect host. *Galleria mellonella* larvae were infected individually in 55×20 mm petri dishes lined with filter paper (Whatman #1). Each insect host received a dose of approximately 200 nematodes in 0.5 ml of deionized water. Infected hosts were kept at 26°C, and transferred to 'White traps' after host death (approximately 2 days). White traps were constructed by placing a 15×10 mm petri dish face-down inside a 55×20 mm petri dish filled with 15 ml of deionized water and laying a piece of filter paper (55 mm) over the smaller dish. Hosts were placed on the filter paper and monitored for nematode emergence starting one week after infection.

Ten replicate lines of two experimental treatments were established with the original mixture from the three sources. In the high-migration treatment, 20 hosts were infected per line. Of the subset of these hosts that produced nematodes during the first 3 days of emergence, eight hosts were chosen at random. For each host, infective juvenile nematodes that emerged in the first 48 h of emergence were collected by first discarding the host and then rinsing the White trap with deionized water to a total volume of 50 ml. Twenty millilitres of this nematode suspension per host was added to a beaker to make a mixture of infective juveniles produced by the eight hosts. This mixture was used to infect a different set of hosts for the next round of infection. All 20 hosts in a line were infected as described above with a dose of 200 nematodes from this mixture.

In the low-migration treatment, eight sub-lines were established within each of the 10 replicate lines. For each sub-line, three hosts were infected identically. One host per sub-line was chosen at random and emerging nematodes were collected as in the high-migration treatment. In addition to a mixture of the infective juveniles produced by all eight hosts, 20 ml of the nematode suspension was reserved separately in a petri dish for each sub-line. New hosts were infected with a dose of 180 nematodes from the sub-line and 20 nematodes from the mixture, in a manner otherwise identical to the high-migration treatment (Fig. 1).

The experiment was conducted as two blocks (five lines of each treatment per block) for 20 rounds of infection over the course of one year (2004–2005). Infective juveniles were
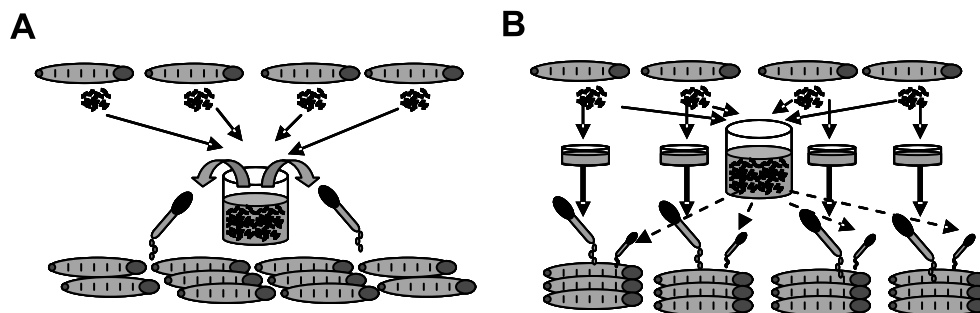

Fig. 1. Schematic representation of the infection protocol for (A) the high-migration treatment and (B) the low-migration treatment. In the high-migration treatment, infective juvenile nematodes emerging from eight hosts are mixed and used to infect the next generation of hosts. In the low-migration treatment, a given host receives 90% of its infective dose from nematodes emerging from a single host and 10% from the pooled contributions of eight hosts. In the schematic, for clarity, only half the hosts are shown.

stored in the aqueous suspensions at 4°C between rounds of infection. *Galleria mellonella* larvae were obtained from several different sources over the course of the experiment. In a given round of infection and block, each treatment was always infected with *Galleria* that had been obtained from the same source and treated similarly prior to infection.

Analyses of host exploitation

For each host passage, we compared the number of infective juveniles that emerged from the high-migration and low-migration treatments by counting the number of infective juveniles in the mixture of eight hosts. We compared the average number of infective juveniles produced by each line with a mixed-model, repeated-measures analysis of covariance using the Mixed Procedure in SAS (Littell *et al.*, 1996; Milliken and Johnson, 2002). Each block and line was treated as a random factor, while treatment was considered a fixed, between-subjects effect. The average weight of the eight hosts in each line and the length of time infective juveniles were stored between infections were covariates, while passage was a within-subject effect.

For one block of lines in passage 17, we also followed the emergence of eight individual hosts per line for a total of 112 days post-emergence. We compared the log number of infective juveniles produced per day between treatments with a mixed-model, repeated-measures analysis of covariance. Each host and line was treated as a random subject, while treatment was considered a fixed, between-subjects effect. The weight of the host was a covariate and the day post-emergence was a within-subject effect.

We also examined the effect of treatment on day of infective juvenile emergence over the time course of the experiment. We performed a repeated-measures analysis using the GLIMMIX macro of SAS, which allowed us to incorporate the categorical nature of the dependent variables as well as the random effects of block and line (Littell *et al.*, 1996). We assumed that the errors were Poisson distributed. Treatment was considered a between-subjects effect, host mass and the length of time infective juveniles were stored between infections were considered covariates, and passage was considered a within-subject effect.

Analyses of host mortality

Due to the unexpected results obtained from our analysis of day of infective juvenile emergence, we also examined the effect of treatment on host mortality rate. As part of the transfer of hosts from their infection plates to White traps, we recorded which hosts were alive and therefore not transferred. We tested whether the probability of a host being dead on the day of transfer to White traps was affected by the treatment or passage using a repeated-measures logistic regression analysis using the GLIMMIX macro of SAS. Treatment was considered a between-subjects effect, while host mass, the length of time infective juveniles were stored between infections, the day of mortality census (transfers to White traps occurred 2–6 days post infection), and passage were considered within-subject effects.

We also performed three, independent assays of host mortality using newly emerged infective juveniles from passage 21 of one block of lines ($n = 5$ lines per treatment). In each assay, 20 hosts were individually exposed to 200 infective juveniles from one experimental line for a total of 200 hosts per assay. In the low-migration treatment, we exposed hosts to

nematodes that emerged from a single host chosen at random from each of the five lines, while in the high-migration treatment each host was exposed to nematodes that emerged from eight hosts. Hosts were examined after 27 h (when mortality was between 50 and 80%) and categorized as alive (clearly moving or responding to a probe), non-responsive, or flaccid (indicative of septicaemia and host tissue degradation). We used a Mantel-Haenszel Mean Score Statistic to control for the different assays and to test the hypothesis that hosts infected with nematodes from the low-migration treatment transitioned from alive to non-responsive then flaccid more quickly than hosts infected with nematodes from the high-migration treatment (Stokes *et al.*, 1995).

RESULTS

Over the time course of the experiment, we saw no consistent differences between low-migration and high-migration treatments in the number of infective juveniles produced in the first 48 h of emergence (Fig. 2A, Table 1). There was considerable variation between passages in the number of infective juveniles produced, but there were no significant differences in how the treatments responded across passages (Table 1). More infective juveniles emerged from larger hosts, while fewer emerged as the duration of storage between infections increased (Table 1). There were no significant interactions between these covariates and the main effects of treatment or passage.

Over the time course of an individual infection, examined at passage 17, there were no differences between low-migration and high-migration treatments in the number of infective juveniles emerging per day (Fig. 2B, Table 2). The number of emerging juveniles dropped off dramatically with day, but this decline did not vary significantly with treatment (Table 2). Larger hosts produced more infective juveniles, and this effect did not vary significantly with treatment or day.

The effect of treatment on the day of infective juvenile emergence varied significantly over the time course of the experiment (Fig. 3A, Table 3). Before passage 7 there was no divergence between treatments. In passages 8 and 9 nematodes emerged significantly earlier in the high-migration treatment, but this difference did not persist in passages 10–13. As of passage 14, nematodes emerged consistently earlier in the low-migration treatment ($F_{1,17} = 14.10$, $P = 0.0016$). Day of emergence was also later when hosts were larger and when infective juveniles were stored longer prior to infection (Table 3). There were no significant interactions between these covariates and treatment.

The effect of treatment on host mortality also varied significantly over the time course of the experiment (Fig. 3B, Table 3). Mortality rate fluctuated from passage to passage; however, whenever the two treatments differed significantly (in passage 12, 18, and 19), hosts infected with low-migration nematodes had higher mortality rates than those infected with high-migration nematodes. Host mortality rate decreased with the duration of nematode storage and increased with the day of census, but was not significantly affected by host mass (Table 3). There were no significant interactions between these covariates and the treatments.

The mortality assays performed with passage 21 nematodes confirmed the mortality differences seen during the time course of the experiment. All three replicate assays showed the same results: the progression of host morbidity and mortality was significantly faster with hosts infected with nematodes from the low-migration treatment ($X^2_{SMH} = 11.55$, $P = 0.0007$) (Fig. 4). In each case, hosts infected with nematodes from the low-migration
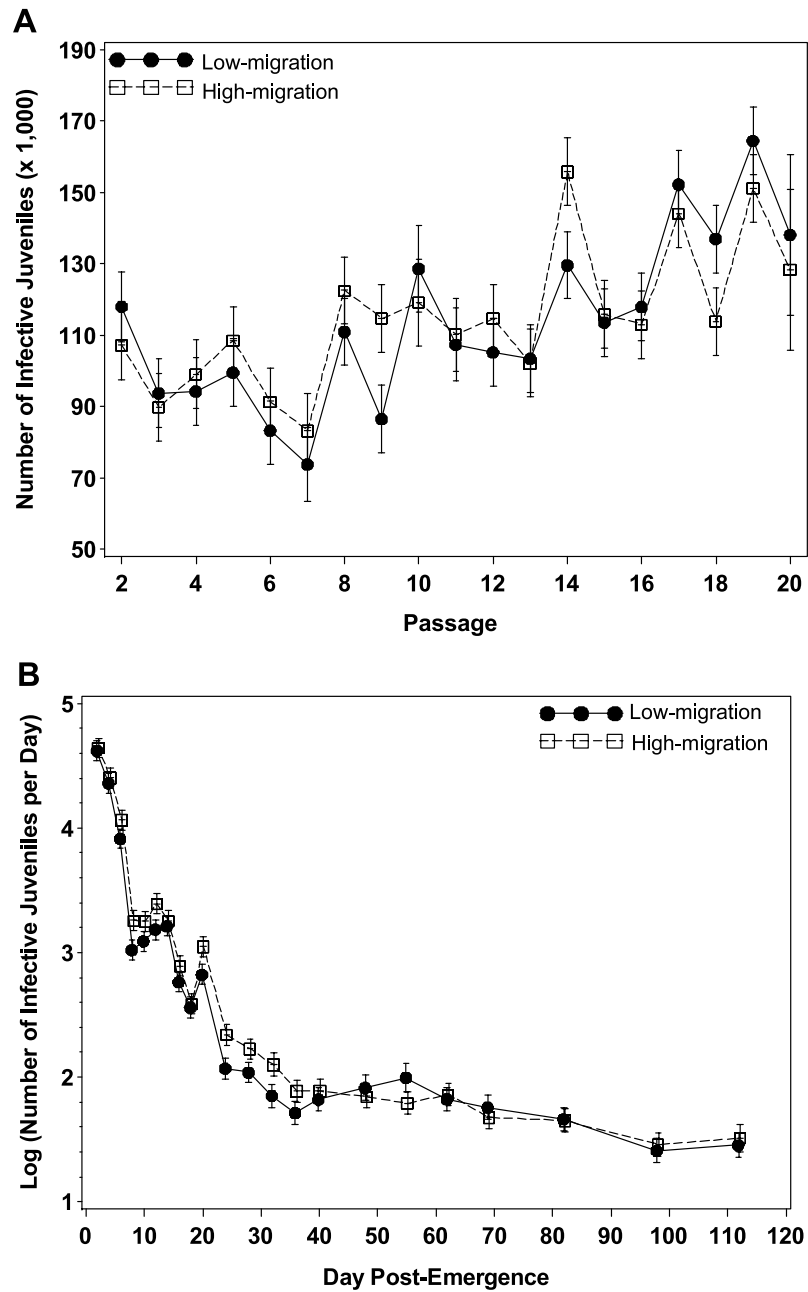
A

B

Fig. 2. The least square mean number (±1 standard error) of infective juveniles emerging from nematodes of each treatment from (A) repeated-measures analysis of covariance over the time course of the experiment or (B) repeated-measures analysis of covariance over the time course of a single infection at passage 17.

Table 1. Results of fixed effects from a mixed-model, repeated-measures analysis of covariance on the average number of emerging infective juveniles over the time course of the experiment

Source of variation	Degrees of freedom	F	P
Migration treatment	1,17	0.13	0.7196
Host mass	1,318	58.06	<0.0001
Storage duration	1,318	7.48	0.0066
Passage	18,318	9.84	<0.0001
Treatment × passage	18,318	1.13	0.3195

Table 2. Results of fixed effects from a mixed-model, repeated-measures analysis of covariance on the average number of emerging infective juveniles from an individual host over the time course of an infection

Source of variation	Degrees of freedom	F	P
Migration treatment	1,8	1.68	0.2313
Host mass	1,1363	55.21	<0.0001
Day	21,1363	345.59	<0.0001
Treatment × day	21,1363	1.22	0.2268

Table 3. Results of fixed effects from mixed-model, repeated-measures analyses of the day of infective juvenile emergence and host mortality rate

Source of variation	Day of emergence			Host mortality		
	Degrees of freedom	F	P	Degrees of freedom	F	P
Migration treatment	1,17	0.79	0.3869	1,17	2.89	0.1075
Host mass	1,6180	20.47	<0.0001	1,6326	2.84	0.0921
Storage duration	1,6180	253.20	<0.0001	1,6326	320.92	<0.0001
Day of census	—	—	—	1,6326	4.71	0.0300
Passage	18,6180	46.68	<0.0001	15,6326	6.90	<0.0001
Treatment × passage	18,6180	5.30	<0.0001	15,6326	2.62	0.0006

treatment were almost twice as likely to be flaccid as hosts infected with high-migration nematodes.

DISCUSSION

We predicted that nematodes from the low-migration treatment would evolve a lower reproductive rate relative to nematodes from the high-migration treatment. Our rationale was that over the course of the experiment, as relatedness increased in the low-migration lines, kin selection would favour more prudent host exploitation. However, none of our three
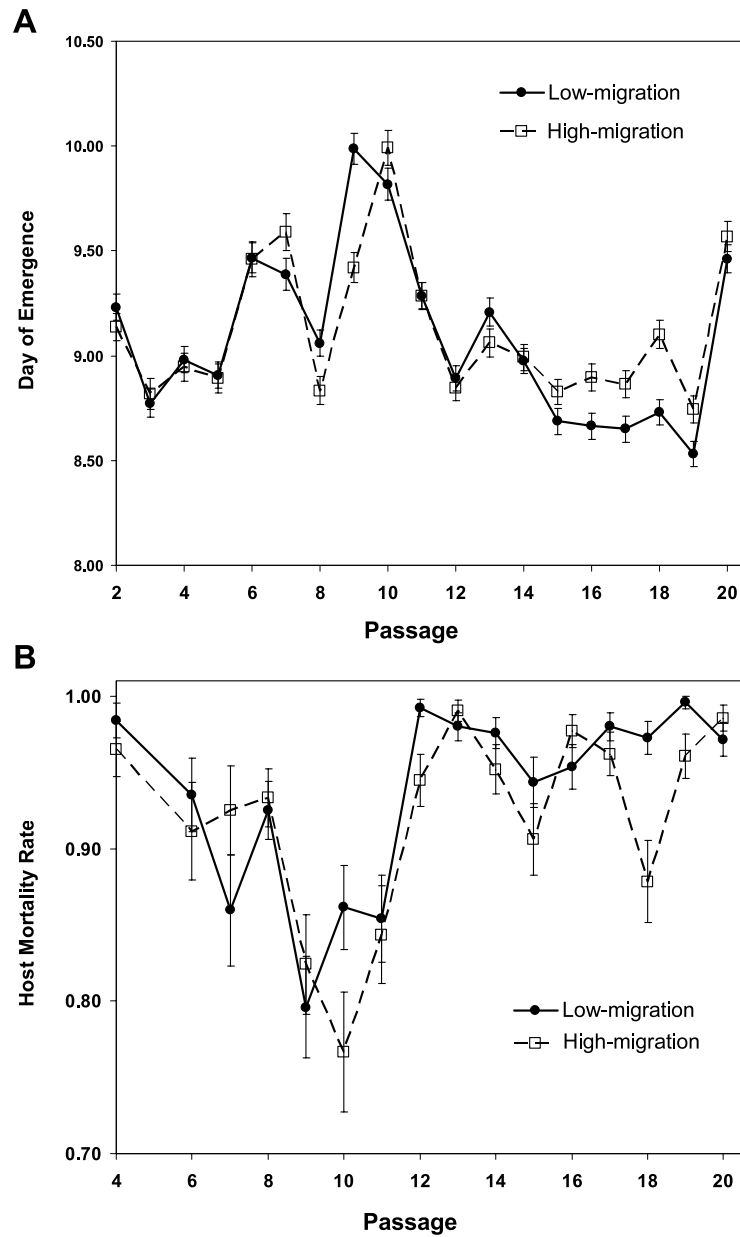
Fig. 3. The estimated mean (±1 standard error) day of infective juvenile emergence (A) and host mortality rate (B) for each treatment from repeated-measures analyses over the time course of the experiment.

measures of host exploitation supported this prediction. Specifically, there was no difference between treatments in the number of infective juveniles that emerged in either the first 48 h from a host (Table 1, Fig. 2A) or over the entire time course of infection (Table 2, Fig. 2B). In addition, opposite to expectation, we found that nematodes from the
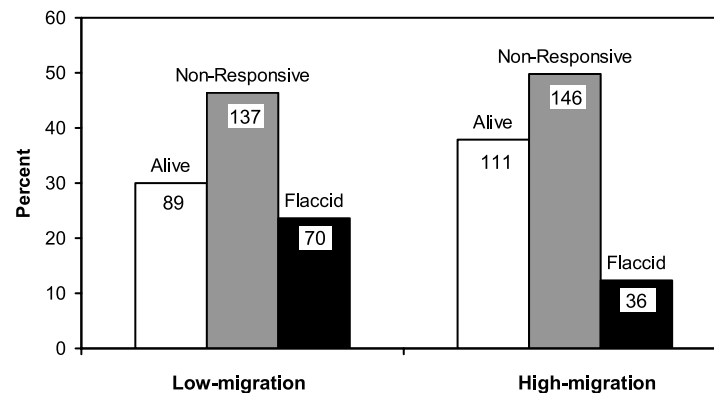
Fig. 4. Percentages of hosts categorized as 'alive' (clearly moving or responding to a probe), 'non-responsive', and 'flaccid' (characteristic of septicaemia and host tissue degradation) 27 h after infection with nematodes from the low-migration or high-migration line. Data shown here are pooled from three replicate assays; in each, 200 hosts were infected with nematodes from passage 21.

low-migration treatment emerged significantly earlier than those in the high-migration treatment (Table 3, Fig. 3A). In what follows, we discuss several possibilities that might explain these findings.

We assumed that nematodes that exploit the host at a faster rate would reach higher population densities within the host, and release more infective juveniles earlier in the course of an infection. Our results provided no evidence for an evolutionary shift in the number of infective juveniles produced between the low-migration and high-migration treatments once emergence began. A possible reason for this lack of response could be a lack of heritable genetic variation for the number and timing of infective juveniles produced. However, genetic variability in the number of infective juveniles produced has been documented among populations of *S. carpocapsae* (Somasekhar *et al.*, 2002), and direct selection has been effective in shifting the timing of emergence in *S. glaseri* (Stuart *et al.*, 1996).

It is also possible that our experiment did not impose strong enough selection (relative to drift) between treatments on the number or timing of infective juveniles produced. Our prediction that the high-migration lines would evolve higher rates of reproduction was based on the assumption that greater early reproduction within the host comes at a cost of lower transmission success. High-migration lines are predicted to bear this higher cost because of the greater within-host competition for access to the infective pool (Frank, 1996). It is possible that because nematodes were placed directly on their insect host in our experiment, we removed an important among-host selective mechanism that might have favoured fewer, higher quality nematodes emerging in the low-migration treatment.

Nevertheless, we did observe a small shift in the day of emergence between treatments, and the direction of this shift (low-migration emerging before high-migration) was exactly the opposite to that of our prediction (Fig. 3A). However, our prediction assumed indirect interactions (via exploitation competition) among parasites within the host; in retrospect, this assumption might not have been met. In a seminal empirical study, Turner and Chao (1999) showed that host exploitation can increase with relatedness, if parasites interact in exploiting their host (Brown *et al.*, 2002; Foster, 2005). For example, if parasites 'cooperate' by producing a shared product that aids in the exploitation of the host (e.g. iron-scavenging

siderophores in bacteria), then selection favours the evolution of selfish parasites that freeload by not producing the product, because they still garner the benefit, but pay none of the cost of production. Thus, unrelated parasites would evolve lower host exploitation than related parasites (Chao *et al.*, 2000; West and Buckling, 2003; Griffin *et al.*, 2004). On the other hand, if parasites directly interfere with one another, for example by producing a chemical that harms conspecific parasites, but not clone-mates (e.g. bacteriocins), then unrelated strains may interfere with each other also leading to lowered host exploitation (Gardner *et al.*, 2004).

While we believe that the nematodes compete via exploitative competition within the host (Selvan *et al.*, 1993), it is possible that there could be cooperation and freeloading among nematodes in the production of insecticidal toxins (Goetz *et al.*, 1981; Burman, 1982; Simoes, 2000). Greater production of insect toxins could explain the quicker host death (Figs. 3B and 4) and faster exploitation of the host (Fig. 3A) observed in the low-migration lines. To date there is no evidence to support this hypothesis. In contrast, the symbiotic bacteria of our nematode, *Xenorhabdus nematophila*, is known to produce bacteriocins (Thaler *et al.*, 1995). Additionally, using *X. nematophila* and another species of nematode-associated bacteria, Massey and colleagues (2004) found that, when two bacteriocin-producing strains infected the same host, they each inhibited the other's growth and reduced host mortality. Hence, in our experiment, the bacteria could have evolved in response to the migration treatments. Specifically, the high-migration lines may have favoured greater bacteriocin activity compared with the low-migration lines, which could have had the effect of both reducing the rate of host mortality (Figs. 3B and 4), and in delaying the emergence of infective juveniles in later passages of the experiment (Fig. 3A).

In summary, none of our three measures of host exploitation by the nematodes supported the prediction that nematodes from the high-migration treatment would have a higher rate of host exploitation than nematodes from the low-migration treatment. In fact, nematodes from the high-migration treatment took longer to emerge from their hosts, indicating a slower rate of host exploitation. This result, in addition to the faster mortality in the low-migration lines, hints that either the bacteria or the nematodes evolved differences in toxin production in response to our migration treatments.

ACKNOWLEDGEMENTS

REFERENCES

Anderson, R.M. and May, R.M. 1982. Coevolution of hosts and parasites. *Parasitology*, **85**: 411–426.

Boemare, N. and Akhurst, R.J. 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (Enterobacteriaceae). *J. Gen. Microbiol.*, **134**: 751–761.

Boots, M. and Mealor, M. 2007. Local interactions select for lower pathogen infectivity. *Science*, **315**: 1284–1286.

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

Brown, S.P., Hochberg, M.E. and Grenfell, B.T. 2002. Does multiple infection select for raised virulence? *Trends Microbiol.*, **10**: 401–405.

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

Chao, L., Hanley, K.A., Burch, C.L., Dahlberg, C. and Turner, P.E. 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q. Rev. Biol.*, **75**: 261–275.

Davies, C.M., Fairbrother, E. and Webster, J.P. 2002. Mixed strain schistosome infections of snails and the evolution of parasite virulence. *Parasitology*, **124**: 31–38.

Dunphy, G.B. and Webster, J.P. 1988. Lipopolysaccharides of *Xenorhabdus nematophilus* (Enterobacteriaceae) and their haemocyte toxicity in non-immune *Galleria mellonella* (Insecta: Lepidoptera) larvae. *J. Gen. Microbiol.*, **134**: 1017–1028.

Foster, K.R. 2005. Hamiltonian medicine: why the social life of pathogens matters. *Science*, **308**: 1269–1270.

Frank, S.A. 1992. A kin selection model for the evolution of virulence. *Proc. R. Soc. Lond. B*, **250**: 195–197.

Frank, S.A. 1996. Models of parasite virulence. *Q. Rev. Biol.*, **71**: 37–78.

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

Gaugler, R. 2002. *Entomopathogenic Nematology*. Wallingford, UK: CABI Publishing.

Gingery, R.E. and Nault, L.R. 1990. Severe maize chlorotic dwarf disease caused by double infection with mild virus strains. *Phytopathology*, **80**: 687–691.

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

Griffin, A.S., West, S.A. and Buckling, A. 2004. Cooperation and competition in pathogenic bacteria. *Nature*, **430**: 1024–1027.

Han, R. and Ehlers, R. 2000. Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic *in vivo* conditions. *J. Invertebr. Pathol.*, **75**: 55–58.

Herre, E.A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science*, **259**: 1442–1445.

Kerr, B., Neuhauser, C., Bohannan, B.J.M. and Dean, A.M. 2006. Local migration promotes competitive restraint in a host–pathogen 'tragedy of the commons'. *Nature*, **442**: 75–78.

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

Littell, R.C., Millikin, G.A., Stroup, W.W. and Wolfinger, R.D. 1996. *SAS System for Mixed Models*. Cary, NC: SAS Institute.

Lively, C.M. 2005. Evolution of virulence: coinfection and propagule production in spore-producing parasites. *BMC Evol. Biol.*, **5**: 64.

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

May, R.M. and Anderson, R.M. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. *Proc. R. Soc. Lond. B*, **219**: 281–313.

May, R.M. and Nowak, M.A. 1995. Coinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B*, **261**: 209–215.

Milliken, G.A. and Johnson, D.E. 2002. *Analysis of Messy Data, Vol. III: Analysis of Covariance*. Boca Raton, FL: Chapman & Hall/CRC Press.

Nguyen, K.B. and Smart, G.C.J. 1995. Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *J. Nematol.*, **27**: 206–212.

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

Pickering, J., Read, A.F., Guerrero, S. and West, S.A. 2000. Sex ratio and virulence in two species of lizard malaria parasites. *Evol. Ecol. Res.*, **2**: 171–184.

Qui, L. and Bedding, R. 2000a. Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. *Nematology*, **2**: 551–559.

Qui, L. and Bedding, R.A. 2000b. Energy metabolism and survival of the infective juveniles of *Steinernema carpocapsae* under oxygen-deficient conditions. *J. Nematol.*, **32**: 271–280.

Selvan, S., Campbell, J. and Gaugler, R. 1993. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *J. Invertebr. Pathol.*, **62**: 278–284.

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

Somasekhar, N., Grewal, P.S. and Klein, M.G. 2002. Genetic variability in stress tolerance and fitness among natural populations of *Steinernema carpocapsae. Biol. Control*, **23**: 303–310.

Stokes, M.E., Davis, C.S. and Koch, G.G. 1995. *Categorical Data Analysis Using the SAS System*. Cary, NC: SAS Institute Inc.

Stuart, R.J., Lewis, E.E. and Gaugler, R. 1996. Selection alters the pattern of emergence from the host cadaver in the entomopathogenic nematode, *Steinernema glaseri. Parasitology*, **112**: 183–189.

Taylor, L.H., Mackinnon, M.J. and Read, A.F. 1998. Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi. Evolution*, **52**: 583–591.

Thaler, J.O., Baghdiguian, S. and Boemare, N. 1995. Purification and characterization of xenorhabdicin, a phage tail-like bacteriocin, from the lysogenic strain F1 of *Xenorhabdus nematophilus. Appl. Environ. Microbiol.*, **61**: 2049–2052.

Turner, P.E. and Chao, L. 1999. Prisoner's dilemma in an RNA virus. *Nature*, **398**: 441–443.

van Baalen, M. and Sabelis, M.W. 1995. The dynamics of multiple infection and the evolution of virulence. *Am. Nat.*, **146**: 881–910.

West, S.A. and Buckling, A. 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. Lond. B*, **270**: 37–44.