doi:10.1111/j.1420-9101.2008.01576.x

Low migration decreases interference competition among parasites and increases virulence

F. VIGNEUX,*,[1] F. BASHEY,*,[1] M. SICARD† & C. M. LIVELY*

*Department of Biology, Indiana University, Bloomington, IN, USA
†Laboratoire Ecologie, Evolution, Symbiose, Universite de Poitiers, UMR CNRS 6556, Poitiers, France

Keywords:

bacteriocin;
evolution of virulence;
growth inhibition;
kin selection;
parasite migration rate;
Xenorhabdus nematophila.

Abstract

Competition among different parasite genotypes within a host is predicted to affect virulence. The direction of this effect, however, depends critically on the mechanisms that parasites use to compete or to cooperate with each other. One mechanism that bacteria use to compete with each other is via the production of bacteria-killing toxins, called bacteriocins. This warfare among parasites within a host is predicted to reduce the rate of host exploitation, resulting in lower virulence. By contrast, if parasites within a host are highly related, there could be a reduction in within-host conflict, increasing virulence. We examined this idea by allowing an insect-parasitic nematode (*Steinernema carpocapsae*) and its symbiotic bacteria (*Xenorhabdus nematophila*) to evolve for 20 passages under two different migration treatments (low and high). We found that host mortality rates were higher in the low-migration treatment when compared with the high-migration treatment. In addition, bacteria isolated from the same insect host inhibited each other's growth, but only in the high-migration treatment. These results show that population structure and interactions among parasites within hosts can be critical to understanding virulence.

Introduction

The expected relationship between the genetic similarity of co-infecting parasites (relatedness) and the detrimental effect they cause their host (virulence) has undergone a paradigm shift in recent years (Brown *et al.*, 2002; Foster, 2005). The long-standing prediction was that when parasites co-infecting a host are highly related, kin selection should favour prudent host exploitation and reduced virulence relative to a co-infection comprised of unrelated parasites (Hamilton, 1972; Frank, 1992, 1994, 1996). The reasoning being that within-host competition would drive unrelated parasites to overexploit their host, leading to a 'tragedy of the commons' and greater virulence. This view assumes that the competition among parasite strains is mediated through resource utilization. However, several recent studies have shown that parasites can engage in other forms of interactions (Turner & Chao, 1999; Griffin *et al.*, 2004; Massey *et al.*, 2004). For example, parasites may cooperate in attacking their host by producing a public good, which may benefit individuals other than the producing parasite. In this case, virulence increases rather than decreases with high relatedness as fewer freeloaders are maintained (Chao *et al.*, 2000; West & Buckling, 2003). Alternatively, resource competition among co-infecting parasites can lead to interference competition or antagonistic interactions between them, which can also affect the relationship between virulence and relatedness (Gardner *et al.*, 2004).

One of the most intriguing means of interference competition is a form of chemical warfare engaged in by bacteria. All lineages of bacteria have a diverse arsenal of weapons, called bacteriocins, which are bacteriocidal toxins thought mainly to affect closely related strains and species (Riley & Chavan, 2007). Individual clones are immune to their own bacteriocin, but they pay a cost for production, as lysis is often involved in bacteriocin release (Riley & Gordon, 1999). Despite their ubiquity, the roles of bacteriocins in nature are virtually unknown. While they have long been assumed to be important in intraspecific competition, only recently has it been

Correspondence: F. Bashey, Department of Biology, Indiana University, 1001 E Third St, Jordan Hall, Bloomington, IN 47405, USA.
Tel.: +1 812 855 3282; fax: + 812 855 6705;
e-mail: fbasheyv@indiana.edu

[1]These authors contributed equally to the work.

demonstrated that colicins (bacteriocins produced by *Escherichia coli*) are effective mediators of intraspecific competition *in vivo* (Kirkup & Riley, 2004). Additionally, some bacteriocins are involved in quorum sensing (Gillor, 2007), and evidence suggests a possible role in community-level interactions (Tait & Sutherland, 2002; Riley *et al.*, 2003).

Bacteriocins are also thought to influence virulence. Massey *et al.* (2004) showed that host death rate was lower when hosts were co-infected with two different genera of bacteria, which were capable of inhibiting each other's growth *in vitro*. They suggested that bacteriocin-based interactions might cause virulence in mixed infections to be lower than in single-strain infections. Moreover, a phenotypic model developed by Gardner *et al.* (2004) predicted that allocation to bacteriocin production, and hence virulence, might evolve in response to the average relatedness of con-specific competitors. These studies motivated us to examine the link between relatedness, virulence and bacteriocin activity.

We manipulated parasite population structure to examine its effect on virulence evolution, using the insect-parasitic nematode *Steinernema carpocapsae* and its symbiotically associated bacteria *Xenorhabdus nematophila*. This species of bacteria produces a wide array of insect and microbial toxins (Forst & Nealson, 1996) as well as a phage tail-like bacteriocin called xenorhabdicin. Xenorhabdicin has been shown to inhibit the growth of other *Xenorhabdus* species and some species in closely related genera (Boemare *et al.*, 1992; Thaler *et al.*, 1995; Sicard *et al.*, 2005), but no intraspecific inhibition has been previously shown.

In our study, we allowed parasites (nematodes and their bacteria) to evolve under two different migration treatments. In the low-migration treatment, parasites had a 90% probability of co-infecting a host with parasites emerging from the same insect host in the previous passage; whereas, in the high-migration treatment, parasites were mixed at random with every passage. Our previous work on these experimental lines, which focused on the nematodes, indicated faster host exploitation in the low-migration treatment (Bashey *et al.*, 2007). As both the nematodes and the bacteria produce insect toxins, and both potentially engage in interference competition within the host, faster host exploitation and greater virulence in the low-migration treatment could arise from a greater contribution to insect-toxin production or due to less fighting among the parasites. Here, we focus on the role of antagonistic interactions among co-infecting bacteria. Thus, we compared the treatments, after 20 passages through insect hosts, for differences in virulence and in the level of inhibition observed between bacterial clones isolated from infected insects. We found reduced rates of mortality of insect hosts, and higher levels of inhibition among bacteria, when insects were infected with parasites from the high-migration treatment.

Materials and methods

Parasite life cycle and source

Infective juveniles of *S. carpocapsae* are free-living, non-feeding and developmentally dormant in the soil (Poinar & Leutenegger, 1968). Each infective juvenile nematode carries approximately 100 *X. nematophila* cells in a specialized part of its intestine (Martens *et al.*, 2003; Sicard *et al.*, 2003). Upon entering an insect host, the nematode releases its bacteria and resumes development (Poinar, 1966). Both the nematode and the bacteria contribute to killing the insect (Burman, 1982; Dunphy & Webster, 1988; Simoes, 2000) and each species reproduces separately inside the insect carcass. *Xenorhabdus nematophila* contributes to nematode fecundity (Poinar & Thomas, 1966; Han & Ehlers, 2000; Sicard *et al.*, 2003) and protects the insect carcass from other organisms (Thaler *et al.*, 1997; Zhou *et al.*, 2002; Sicard *et al.*, 2005). When resources become limiting (Popiel, 1989), the juvenile nematodes become colonized by one or two bacteria cells (Martens *et al.*, 2003) and emerge from the insect.

Steinernema carpocapsae infects a broad range of insects (Peters, 1996). In our study, we used the greater wax moth caterpillar, *Galleria mellonella*, as the insect host. Parasites (the nematode *S. carpocapsae* and its symbiotically associated bacteria *X. nematophila*) were obtained from three commercial sources: Integrated Biocontrol Systems Inc. (Greendale, IN, USA: *S.c.* strain 'Sal'), Mellinger's Inc. (N. Lima, OH, USA: *S.c.* an unidentified strain) and Biocontrol Network (Brentwood, TN, USA: *S.c.* strain 'All') and equal concentrations of each were combined to create the initial parasite source population.

Experimental lines

From this initial source population, 10 experimental lines were established and separately maintained from each other for 20 host passages. In each passage, parasites were propagated by one of two migration treatments through a novel set of the insect host. In the five lines comprising the high-migration treatment, parasites were propagated by infecting a given host with a mixture of parasites that emerged from eight hosts. Each insect was placed in a 55×20 mm^2 Petri dish lined with filter paper and infected with 200 nematodes in 0.5 mL of de-ionized water. Infected hosts were kept at 26 °C and transferred to modified White traps for collection of emerging parasites (Bashey *et al.*, 2007). In the five lines comprising the low-migration treatment, 90% of the infective dose came from one host and 10% came from a mixture of eight hosts. Thus, in both treatments, parasites were propagated through eight hosts with each passage;

however, the treatments differed in the probability that parasites emerging from a given host would co-infect a new host together. Within the low-migration lines, this protocol created eight sublines that represent groups of parasites that were kept together each passage.

Mortality assay

After 20 experimental passages, we compared the insect mortality rates of the low- and high-migration treatments (Fig. 1). Two hundred *G. mellonella* were infected with parasites (nematodes with their bacteria) from each experimental line and checked for mortality eight times over 72 h postinfection. Each caterpillar was infected in a manner similar to how the experimental lines were propagated: using a dose of 200 nematodes that emerged from eight different hosts (high-migration) in the previous passage or only nematodes emerging from a single host (low-migration). Caterpillars were scored as alive if they moved spontaneously or in response to probing. We performed Cox proportional hazards regression with the TPHREG Procedure in SAS/STAT® software (SAS Institute Inc., Cary, NC, USA) to estimate the survival probability at each census and to test for an overall difference in survival curves between treatments while accounting for the nested effect of experimental line.

Bacteria sampling

To assay inhibition among bacterial clones, 20 dead hosts from the mortality assay described above were sampled for bacteria at 66 h postinfection. The two hosts from each high-migration line represent replicate infections, whereas the two hosts from each low-migration line were infected with parasites that emerged from different hosts in the previous passages (Fig. 1). Each dead host was injected with 1 mL of Luria–Bertani broth (LB) and 1 mL of LB, the haemolymph, and the bacteria it contained were collected. After centrifugations, bacteria

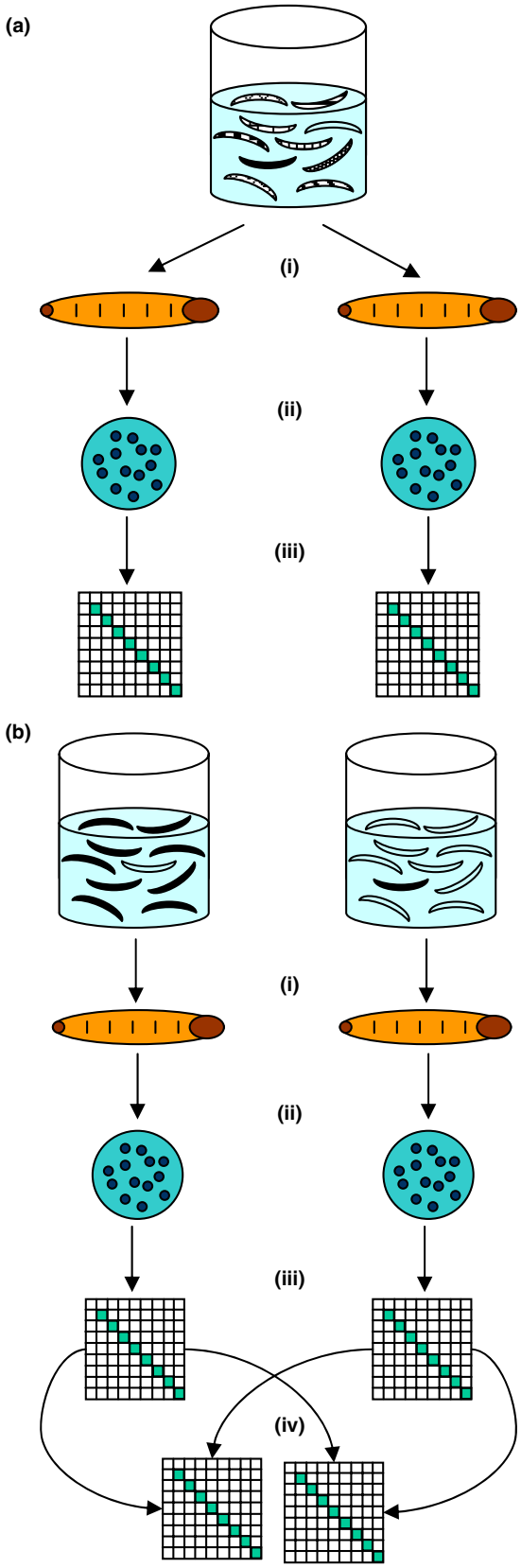

Fig. 1 Schematic of the experiment. (i) Insect hosts were infected with parasites (nematodes and their symbiotic bacteria) from one of five experimental lines from either the (a) high- or (b) low-migration treatment. In the high-migration treatment, parasites emerging from eight hosts were randomly mixed at every passage, whereas, in the low-migration treatment, 90% of the infective dose was comprised of parasites emerging from the same host. A total of 200 insects were infected and observed for mortality. (ii) Bacteria were extracted from two hosts per line and (iii) up to eight bacterial colonies were isolated per host resulting in an 8 × 8 set of within-host inhibition assays for each host. In (a), the two hosts sampled from each high-migration line represent replicate infections. In (b), each sampled host was infected with parasites that emerged from different hosts in previous passages, i.e. two different sublines with each low-migration line. (iv) Between-host inhibition assays tested for inhibition between colonies from these different sublines.

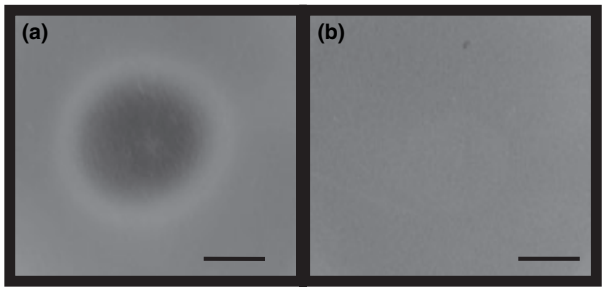

Fig. 2 Photographs showing (a) inhibition or (b) no inhibition of the growth of one bacterial clone caused by the supernatant of another. Line indicates 5 mm.

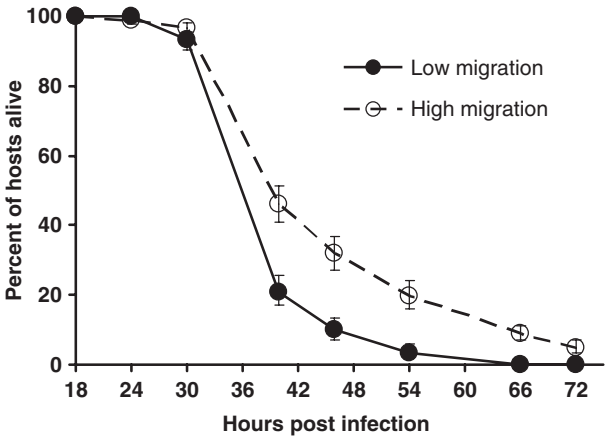

Fig. 3 Average survivorship (±1 SE) for insect hosts infected with parasites (nematodes and their bacteria) from either the low- or high-migration experimental lines. Two hundred *Galleria mellonella* were individually exposed and monitored for 72 h. Survivorship was significantly lower for hosts infected with parasites from the low-migration treatment.

were diluted in LB and plated on nutrient agar supplemented with 0.004% (w/v) triphenyltetrazolium chloride and 0.0025% (w/v) bromothymol blue (NBTA). Up to eight colonies were isolated from each insect host, preserved in glycerol and used in the growth inhibition assays. *Xenorhabdus nematophila* colonies were sampled based on their adsorption of blue dye when grown on NBTA. Species identity was confirmed by sequencing of 16S rDNA (Tailliez *et al.*, 2006).

Growth inhibitions assays

Assays were performed between colonies isolated from the same host (both treatments) and between colonies isolated from different hosts in the same experimental line (low-migration treatment only, see Fig. 1). These clones were grown in liquid culture for 72 h at 28 °C shaking (300 rpm) in 5 mL of LB broth. To test the sensitivity of a clone, molten Soft (MS) agar (0.6% agar) was sowed with 2% (v/v) of its liquid culture. Then, supernatant from the liquid culture of a potential producer clone was filtered through a 0.45-μm membrane, and 15 μL was spotted onto the surface of an MS agar plate containing the potential sensitive clone. Plates were incubated for 48 h, at which time the inhibition of growth of the bacteria in the soft agar below the 15 μL of supernatant could be visualized as a clear zone of inhibition (Fig. 2). Chi-squared tests were performed to see if the total number of inhibitions differed between the sets of inhibition assays.

Results

Hosts infected by parasites (nematodes and bacteria) from the low-migration treatment died significantly earlier than hosts infected by parasites from the high-migration treatment ($\chi^2_1 = 9.52$, $P = 0.002$, Fig. 3). This difference was most pronounced from 40 to 54 h post-infection, where hosts infected by parasites from the high-migration treatment were more than twice as likely to be alive.

Table 1 Comparisons between migration treatments of the within-host inhibition assays.

	Number showing within-host inhibitions	Total examined
Clones*		
Low migration	0	51
High migration	12	49
Cross-tests†		
Low migration	0	294
High migration	19	306
Insect hosts‡		
Low migration	0	8
High migration	4	7
Experimental lines§		
Low migration	0	5
High migration	3	4

*A total of 100 *Xenorhabdus nematophila* colonies were isolated and cross-tested with at least one other clone isolated from the same insect host ($\chi^2 = 14.19$; $P < 0.001$).
†A total of 600 within-host inhibition assays were performed ($\chi^2 = 16.01$; $P < 0.0001$).
‡Multiple clones were successfully isolated from 15 insect hosts (Fisher's exact, $P < 0.0256$).
§Bacteria was successfully isolated from nine of the 10 experimental lines used in the mortality assay (Fisher's exact, $P < 0.0476$).

The higher virulence in the low-migration lines was associated with the absence of inhibition between bacterial clones sampled from the same host (Table 1). On the contrary, in the high-migration treatment, inhibitions were observed between bacterial clones isolated from the same host in 75% of the lines. In addition, almost one-fourth of the clones isolated from the high-

Table 2 Comparisons of between-host inhibitions in the low-migration treatment with within-host inhibitions in the high-migration treatment.

	Number	Total examined
Producer clones*		
Low migration (between host)	20	47
High migration (within host)	12	49
Sensitive clones†		
Low migration (between host)	8	47
High migration (within host)	14	49
Cross-tests‡		
Low migration (between host)	22	256
High migration (within host)	19	306
Experimental lines§		
Low migration (between host)	3	4
High migration (within host)	3	4

*Number of clones capable of inhibiting at least one other clone ($\chi^2 = 3.52$; $P = 0.0605$).
†Number of clones whose growth was inhibited by at least one other clone ($\chi^2 = 1.81$; $P = 0.1783$).
‡Number of inhibitions observed ($\chi^2 = 1.72$; $P = 0.2790$).
§Number of lines showing inhibitions (Fisher's exact; $P = 1.000$).

migration treatment were capable of inhibiting the growth of at least one other clone isolated from the same insect (Table 1). These differences between treatments were highly significant when analysed at the level of clone or cross-test (Table 1) and remained significant when more conservative tests, accounting for the fact that several clones were sampled from the same insect host and experimental line, were performed (Table 1).

Despite the lack of inhibition between clones isolated from the same insect host in the low-migration treatment, these clones still possessed the ability to inhibit the growth of other *X. nematophila*. In fact, when bacteria from one host were cross-tested against bacteria from a host infected with parasites from a different subline (Fig. 1, iv), inhibitions were observed at a level comparable with those found in the high-migration treatment (Table 2).

Discussion

Our results confirm the importance of parasite population structure on patterns of virulence. We observed significantly faster rates of host death (higher virulence) in the low-migration lines than in the high-migration lines (Fig. 3). We also observed significantly fewer inhibitions among bacterial clones isolated from the same host in the low-migration lines (Table 1). Hence, our data suggest that low migration led to increased relatedness among bacteria within a host, reducing interference competition among them, thus increasing their ability to overcome the host.

Within the low migration lines, we also tested for inhibition among bacterial clones isolated from different hosts. We found that such inhibitions were as common as the inhibitions observed among clones isolated from the same hosts in the high-migration treatment (Table 2). This result demonstrates that the low-migration treatment did not result in a loss of the ability to produce a growth-inhibiting agent. Rather, it suggests that the low-migration treatment was effective in structuring the population. Thus, bacteria isolated from the same host were immune to each other's attacks, but they were not immune to attacks of bacteria isolated from other hosts (i.e. different subpopulations) in the same treatment.

Overall, our results suggest that there was variation among bacterial clones at the origin of the experiment, and that this variation became partitioned among hosts differently in the two migration treatments. Most likely it took several experimental passages before the low-migration lines became structured, as no significant differences in host mortality rate were seen across treatments prior to passage 12 (Bashey *et al.*, 2007). Additionally, it is possible that new variation arose during the course of the experiment by mutation or that our treatments resulted in a difference in investment in bacteriocin production (as modelled by Gardner *et al.*, 2004). Unfortunately, we do not currently have the data to address these possibilities. Furthermore, deriving predictions for our migration treatments based on Gardner's model is difficult as low migration can both increase kinship and decrease the scale of competition (Taylor, 1992a,b; Wilson *et al.*, 1992; West *et al.*, 2002), which would impose conflicting selective pressures on investment in bacteriocin production. Nevertheless, our results unambiguously show that differential migration can affect virulence, and the frequency of within-host inhibitions.

Conclusions

Our results show that parasite migration can affect the outcome of interactions between hosts and parasites, even in the absence of changes to host population densities that were critical in other studies (Kerr *et al.*, 2006; Boots & Mealor, 2007). Further, we provide the first documentation of intraspecific growth inhibition in *X. nematophila*. We also show that high parasite migration rates can lead to more inhibitions between bacterial clones isolated from the same host and to reduced host mortality rates. Therefore, this study provides support for the view that bacteriocins play an important role in mediating intraspecific interactions; and underscores the importance of parasite population structure to our understanding of virulence.

Acknowledgments

We thank C. Fuqua, A. Bhasin, A. Countryman, S. Rangel and C. Reynolds for advice and logistical assistance. This research was supported by NSF DEB-0515832 and by the

Indiana METACyt Initiative of Indiana University, funded in part through a major grant from the Lilly Endowment, Inc.

References

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

Boemare, N.E., Boyer-Giglio, M.H., Thaler, J.O., Akhurst, R.J. & Brehelin, M. 1992. Lysogeny and bacteriocinogeny in *Xenorhabdus nematophilus* and other *Xenorhabdus* spp. *Appl. Environ. Microbiol.* **58**: 3032–3037.

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

Brown, S.P., Hochberg, M.E. & 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. & Turner, P.E. 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q. Rev. Biol.* **75**: 261–275.

Dunphy, G.B. & 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.

Forst, S. & Nealson, K. 1996. Molecular biology of the symbiotic pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. *Microbiol. Rev.* **60**: 21–43.

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

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

Frank, S.A. 1994. Kin selection and virulence in the evolution of protocells and parasites. *Proc. R. Soc. B* **258**: 153–161.

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

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

Gillor, O. 2007. Bacteriocins' role in bacterial communication. In: *Bacteriocins: Ecology and Evolution* (M.A. Riley & M.A. Chavan, eds), pp. 135–145. Springer-Verlag, Berlin.

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

Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3**: 193–232.

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

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

Kirkup, B.C. & Riley, M.A. 2004. Antibiotic-mediated antagonism leads to a bacterial game of rock–paper–scissors *in vivo*. *Nature* **428**: 412–414.

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

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

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.

Poinar, G.O.J. & Leutenegger, R. 1968. Anatomy of the infective and normal third-stage juvenile of *Neooplectana carpocapsae* Weiser (Steinernematidae: Nematoda). *J. Parasitol.* **54**: 340–350.

Poinar, G.O.J. & Thomas, G.M. 1966. Significance of *Achromobacter nematophilus* Poinar and Thomas (Achromobacteraceae: Eubacteriales) in the development of the nematode, DD-136 (*Neoplectana* sp. Steinernematidae). *Parasitology* **56**: 385–390.

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

Riley, M.A. & Chavan, M.A. (eds) 2007. *Bacteriocins: Ecology and Evolution*, Springer-Verlag, Berlin.

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

Riley, M.A., Goldstone, C.M., Wertz, J.E. & Gordon, D. 2003. A phylogenetic approach to assessing the targets of microbial warfare. *J. Evol. Biol.* **16**: 690–697.

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

Sicard, M., Tabart, J., Boemare, N.E., Thaler, O. & Moulia, C. 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.

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

Tait, K. & Sutherland, I.W. 2002. Antagonistic interactions amongst bacteriocin-producing enteric bacteria in dual species biofilms. *J. Appl. Microbiol.* **93**: 345–352.

Taylor, P.D. 1992a. Altruism in viscous populations – an inclusive fitness model. *Evol. Ecol.* **6**: 352–356.

Taylor, P.D. 1992b. Inclusive fitness in a homogeneous environment. *Proc. R. Soc. B* **249**: 299–302.

Thaler, J.O., Baghdiguian, S. & 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.

Thaler, J.O., Boyer-Giglio, M.H. & Boemare, N.E. 1997. New antimicrobial barriers produced by *Xenorhabdus* spp. and *Photorhabdus* spp. to secure the monoxenic development of entomopathogenic nematodes. *Symbiosis* **22**: 205–215.

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

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

West, S.A., Pen, I. & Griffin, A.S. 2002. Cooperation and competition between relatives. *Science* **296**: 72–75.

Wilson, D.S., Pollock, G.B. & Dugatkin, L.A. 1992. Can altruism evolve in purely viscous populations? *Evol. Ecol.* **6**: 331–334.

Zhou, X., Kaya, H.K., Heungens, K. & Goodrich-Blair, H. 2002. Response of ants to a deterrent factor(s) produced by the symbiotic bacteria of entomopathogenic nematodes. *Appl. Environ. Microbiol.* **68**: 6202–6209.

Received 4 April 2008; revised 3 June 2008; accepted 3 June 2008