

Spatial heterogeneity and the stability of host-parasite coexistence

M. A. BROCKHURST*, §, A. BUCKLING† & P. B. RAINEY*, ‡

*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, UK

†Department of Zoology, University of Oxford, South Parks Road, Oxford, UK

‡School of Biological Sciences, University of Auckland, Auckland, New Zealand

§Institut des Sciences de l'Evolution, Université Montpellier II, Place Eugène Bataillon, Montpellier cedex 05, France

Keywords:

bacteriophage;
coevolution;
dispersal;
experimental evolution;
predator-prey;
Pseudomonas aeruginosa;
resistance.

Abstract

Spatially heterogeneous environments can theoretically promote more stable coexistence of hosts and parasites by reducing the risk of parasite attack either through providing permanent spatial refuges or through providing ephemeral refuges by reducing dispersal. In experimental populations of *Pseudomonas aeruginosa* and the bacteriophage PP7, spatial heterogeneity promoted stable coexistence of host and parasite, while coexistence was significantly less stable in the homogeneous environment. Phage populations were found to be persisting on subpopulations of sensitive bacteria. Transferring populations to fresh microcosms every 24 h prevented the development of permanent spatial refuges. However, the lower dispersal rates in the heterogeneous environment were found to reduce parasite transmission thereby creating ephemeral refuges from phage attack. These results suggest that spatial heterogeneity can stabilize an otherwise unstable host-parasite interaction even in the absence of permanent spatial refuges.

Introduction

Spatial heterogeneity is thought to play an important role in determining the dynamics of exploiter–victim interactions (Boots & Sasaki, 1999; Thompson, 1999; Thrall & Burdon, 2002). Spatially heterogeneous environments can theoretically promote more stable coexistence of exploiters and victims by reducing the risk of exploiter attack (Maynard Smith, 1974; Hilborn, 1975; Murdoch, 1977). This can be through providing permanent exploiter free locations (spatial refuges) (Maynard Smith, 1974; Schrag & Mittler, 1996), or through reducing dispersal, which in turn reduces the exploiter–victim encounter rate, thereby creating ephemeral refuges from exploiter attack (Maynard Smith, 1974; Hilborn, 1975; Boots & Sasaki, 1999; Haraguchi & Sasaki, 2000; Hosseini, 2003).

A number of classic studies of predator–prey interactions have found that greater spatial structure can increase the stability of coexistence. Increasing the

viscosity of the culture medium was found to stabilize the otherwise unstable interaction between *Paramecium aurelia* and the predator *Didinium nasutum* (Luckinbill, 1973). Similarly introducing physical barriers into the experimental environment promoted more stable coexistence between the predatory mite *Typhlodromus occidentalis* and the prey mite *Eotetranychus sexmaculatus* (Huffaker, 1958), and between parasitic wasps, *Nasonia vitripennis*, and host flies *Musca domestica* and *Phaenicia sericata* (Pimentel *et al.*, 1963). In each case the key process responsible for coexistence was reduced exploiter dispersal due to increased spatial structure (Hilborn, 1975). More recently, studies have shown that increased habitat structure, whereby habitat is subdivided into a set of interconnected patches (a metapopulation), prolongs coexistence of *D. nasutum* and *Colpidium striatum* (Holyoak & Lawler, 1996; Holyoak, 2000), and the predatory mite *Phytoseiulus persimilis* and the herbivorous mite *Tetranychus urticae* living on bean plants (Ellner *et al.*, 2001). Similarly, increasing metapopulation structure led to more stable coexistence of the host-parasitoid pair, *Callosobruchus chinensis* and *Anisopteromalus clalandrae* (Bonsall *et al.*, 2002).

Populations of bacteria and their viral parasites (bacteriophage) are increasingly being used as model

Correspondence: Michael A. Brockhurst, ISEM, UMII, CC065, Place E Bataillon, 34095 Montpellier Cedex 5, France.
Tel.: 33 467 143667; fax: 33 467 143667;
e-mail: brockhurst@isem.univ-montp2.fr

host-parasite systems. The lifecycle of virulent bacteriophage consists of invasion of bacterial cells, replication, and then lysis of the host cell to release phage progeny; phage therefore impose strong selection for bacterial resistance (Lenski & Levin, 1985). Coexistence requires that some portion of the bacterial population is susceptible to the phage. This can arise through persistent coevolution, where phage continually evolve infectivity to resistant bacteria (Buckling & Rainey, 2002; Brockhurst *et al.*, 2003), or through maintenance of a polymorphic bacterial population containing both resistant and sensitive individuals, where costs associated with resistance allow sensitivity to persist (Lenski & Levin, 1985; Bowers *et al.*, 1994; Bohannan & Lenski, 2000; Brockhurst *et al.*, 2004,2005). Coexistence via the latter mechanism has only been reported for bacteria-phage systems where the culture vessel is maintained for the duration of the experiment; here growth on the walls provides permanent refuge for sensitive bacteria (Chao *et al.*, 1977; Schrag & Mittler, 1996). Experiments where the culture vessels are changed every transfer to eliminate permanent refuges (batch culture) have all reported unstable coexistence, however these experiments were conducted in homogeneous (shaken) microcosms (Schrag & Mittler, 1996; Lythgoe & Chao, 2003).

We present the first explicit investigation into the effect of spatial heterogeneity on the stability of bacteria-phage coexistence in the absence of permanent spatial refuges. Replicate populations of isogenic *Pseudomonas aeruginosa* PAO1 and the bacteriophage PP7 (Olsthoorn *et al.*, 1995) were propagated in homogeneous (constant mixing; shaken) and heterogeneous (unmixed; static) microcosms (Rainey & Travisano, 1998). To prevent the formation of permanent spatial refuges that persist for the whole experiment an aliquot of each population was transferred to a fresh microcosm daily. To test for coexistence, population densities of bacteria and phage were measured every four days. To ascertain the mode of coexistence (i.e. persistent coevolution or the maintenance of a sensitive subpopulation of bacteria), changes in bacterial resistance and phage infectivity were measured through time. To determine the effect of the environmental regimes on parasite dispersal, transmission rates were measured in both homogeneous and heterogeneous microcosms. Finally, the fitness of resistant bacteria was measured in both environments. We hypothesized that spatial heterogeneity would lead to more stable coexistence by reducing dispersal.

Materials and methods

Culturing techniques

Twelve microcosms (30 mL glass universals containing 6 mL of LB broth) were inoculated with 10^6 cells of *P. aeruginosa* PAO1 (grown for 24 h at 37 °C in an orbital shaker at 200 rpm) and 10^5 clonal particles of

bacteriophage PP7. Six populations were propagated at 37 °C in a static incubator (spatially heterogeneous treatment) and six were propagated at 37 °C in an orbital shaker at 200 rpm (spatially homogeneous treatment). After thorough homogenization, an aliquot (60 µL) of each culture was transferred to a fresh microcosm every 24 h, for 24 transfers (approximately 180 bacterial generations). Cultures were frozen and phage isolates taken at every transfer. Densities of bacteria and phage were determined at every fourth transfer by plate counts on LB agar or a soft-agar overlay containing exponentially growing bacteria, respectively.

Growth rate assay in the absence of phage

In order to determine whether the two environments differed in terms of productivity, 36 replicate microcosms were inoculated with 10^6 cells of *P. aeruginosa* PAO1 (grown for 24 h at 37 °C in an orbital shaker at 200 rpm). Half were incubated statically, while the other half were shaken. Populations were destructively sampled after 4, 8 and 24 h and bacterial densities were measured.

Impact on transmission

In order to determine whether increased population mixing increased phage transmission success, 36 replicate microcosms were inoculated with 10^6 cells of *P. aeruginosa* PAO1 (grown for 24 h at 37 °C in an orbital shaker at 200 rpm) and 10^5 clonal particles of bacteriophage PP7. Half were incubated in static microcosms, while the other half were shaken. Populations were destructively sampled after 4, 8 and 24 h and densities of bacteria and phage were measured.

Measurement of bacterial resistance and phage infectivity

Resistance of bacteria (infectivity of phage) was determined by streaking 20 independent bacterial colonies across a line of phage that had previously been inoculated onto an agar plate. A colony was defined as resistant if there was no inhibition of growth; otherwise it was defined as sensitive (Buckling & Rainey, 2002; Brockhurst *et al.*, 2003).

Measurement of coevolution

To determine if antagonistic coevolution occurred, change in the infectivity of phage populations to a bacterial population was measured through time. Specifically, at every fourth transfer, the resistance (proportion resistant colonies) of bacterial populations to past (two transfers previous), contemporary and future (two transfers subsequent) sympatric phage populations was determined. If coevolution was escalatory

(as is commonly observed in coevolving bacteria-phage systems: Bohannan & Lenski, 2000; Buckling & Rainey, 2002), it would be expected, for multiple points, that future phage would be better than contemporary phage, and that contemporary phage be better than past phage at infecting contemporary bacteria, hence a negative slope of bacterial resistance against the time when the phage population was isolated (past, contemporary and future). To determine the rate of coevolution we calculated how much phage infectivity changed between past and future populations, given by the slope of infectivity against time (past, contemporary and future). (Brockhurst *et al.*, 2003).

Individual growth rate experiments in the absence of phage

To test the effect of environmental productivity on the cost of resistance, the growth rates of six independent resistant mutants relative to the ancestor were measured in both environments. A single colony of each derived type, and six colonies of the marked ancestor, were each diluted into 100 μ L of LB. 30 μ L of each was then inoculated individually into one shaken and one static microcosm. Densities were determined at 24 h by plate counts of colonies grown on LB agar. Growth rates were calculated as Malthusian parameters (m), $m = \ln(N_t/N_0)$, where N_0 is the starting density and N_t the final density (Lenski, 1991). Relative fitness (W) was calculated from the ratio of the estimated Malthusian parameters for each derived type relative to the mean of the ancestor.

Results and discussion

Spatial heterogeneity promoted stable coexistence of host and parasite in experimental populations of *P. aeruginosa* and the bacteriophage PP7, while coexistence was less stable in the homogeneous environment. Bacteria persisted for the full course of the experiment in all populations (Fig. 1). Phage populations showed similar patterns in both environments up to day 8, rising to high frequency by day 4 of the experiment before declining. After day 8, however, phage populations in the two environments diverge; in heterogeneous microcosms phage populations stabilize at around day 8 population densities (Fig. 1b, five of six slopes of phage log density against time-point were positive between days 8 and 24), whereas, in homogeneous microcosms all phage populations continue to decline, with one going extinct (Fig. 1a, all six slopes of phage log density against time-point were negative between days 8 and 24; G -test, with Yate's correction, of independence between sign of slope and homogenous vs. heterogeneous environment: $G_1 = 26.3$, $P < 0.01$).

Bacterial populations evolved high levels of resistance to phage by day 4 of the experiment in both environ-

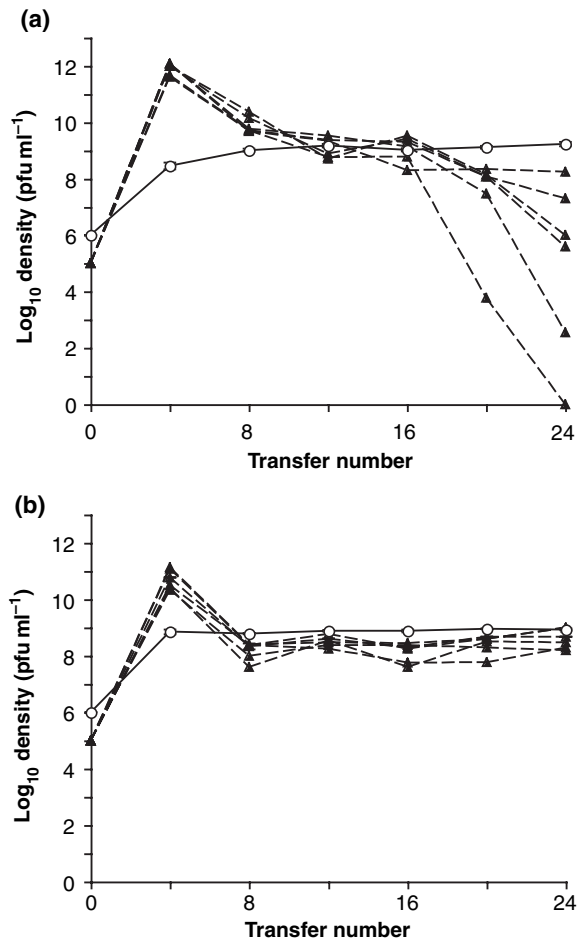


Fig. 1 Densities of phage (closed triangles) and mean \pm SEM densities of bacteria (open circles) under homogeneous (a) and heterogeneous (b) conditions.

ments and remained at high levels in both environments for the course of the experiment (resistance to the ancestral phage averaged over the duration of the experiment; homogeneous environment, 97.3%; heterogeneous environment, 96.8%). However, no significant coevolution, whereby the infectivity of phage populations to a given bacterial population increases through time (Buckling & Rainey, 2002; Brockhurst *et al.*, 2003), was observed in homogeneous or heterogeneous populations (Fig. 2a and b, both environments slopes of infectivity different to zero, Sign-test both $P > 0.2$). Phage populations were therefore likely to be persisting on small subpopulations of sensitive bacteria in both treatments.

Heterogeneous environments are thought to increase the chance of survival for susceptible hosts by providing permanent spatial refuges (Maynard Smith, 1974; Schrag & Mittler, 1996) or through reducing dispersal and hence reducing parasite transmission, creating

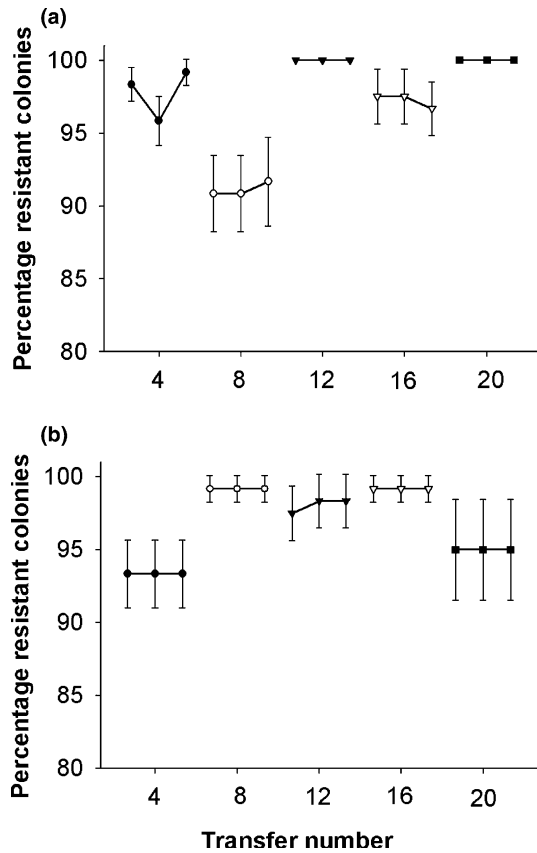


Fig. 2 Rates of coevolution through time in homogeneous (a) and heterogeneous (b) populations of bacteria and phage. Each set of lines shows (from left to right) proportion of bacteria resistant to phage from two transfers in the past, contemporary phage and phage from two transfers in the future. The slope of the line provides a measure of the rate of coevolution over each four-transfer period.

ephemeral refuges (Maynard Smith, 1974; Hilborn, 1975; Boots & Sasaki, 1999; Haraguchi & Sasaki, 2000; Hosseini, 2003). Transferring populations to fresh microcosms every 24 h is likely to have prevented the development of permanent spatial refuges that persist from transfer to transfer. However, greater population mixing in the homogeneous environment (to break up spatial structure) is likely to significantly increase phage transmission. If transmission was greater in the homogeneous environment then phage densities should be higher during the initial stages of phage population growth (the first 24 h). Our data support this (Fig. 3a, density averaged through time; t -test, $t = 3$, d.f. = 5, $P < 0.05$). Although mean bacterial densities were not significantly different during this 24 h period (Fig. 3a, density averaged through time; t -test, $t = 1.37$, d.f. = 9, $P = 0.2$), the reduction in bacterial densities by phage, obtained by comparing bacterial densities with and without phage, was greater in homogeneous environment, providing further support for the hypothesis that

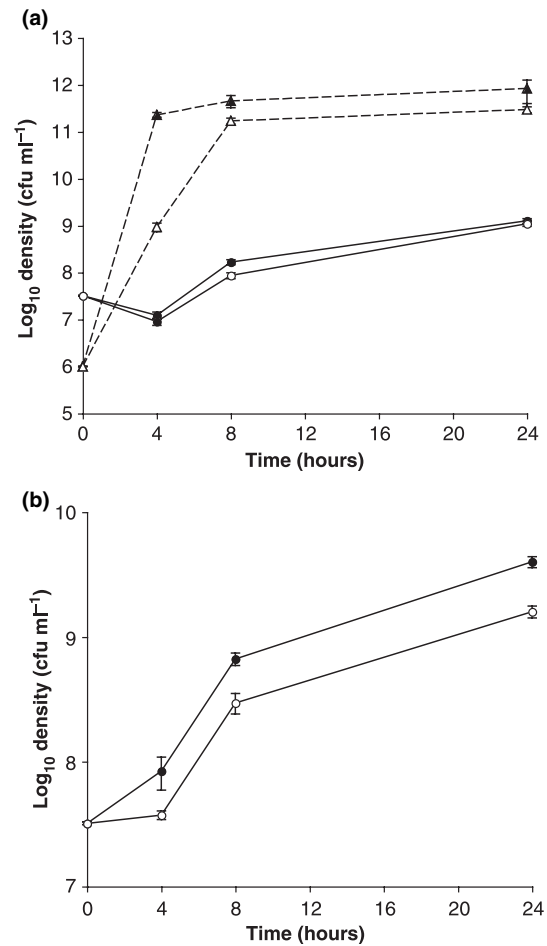


Fig. 3 Mean \pm SEM densities of bacteria (circles) and phage (triangles) under homogeneous (closed symbols) and heterogeneous (open symbols) conditions, in the presence (a) and absence (b) of phage.

mixing increases phage transmission (t -test, $t = 4.37$, d.f. = 7, $P < 0.01$). Increased infection of susceptible hosts by virulent phage through population mixing would by definition have decreased the proportion of susceptible hosts, hence destabilising the coexistence of bacteria and phage. By contrast, spatial heterogeneity is likely to have reduced phage transmission, thereby creating ephemeral refuges from phage attack. This, in turn would allow sensitive bacteria to persist, promoting coexistence. No difference in the average level of resistance was observed between the homogeneous and heterogeneous treatments (bacterial resistance averaged through time not significantly different, data shown above; t -test, $t = 0.08$, d.f. = 7, $P = 0.9$). However, on day 20, during the period of homogeneous phage population decline, there were significantly less sensitive hosts in the homogeneous environment (number of populations containing sensitive bacteria on day

20 greater in the heterogeneous than homogeneous environment; binomial test, $P < 0.05$).

In addition to increasing phage transmission, constant shaking may have altered the environment in other ways that may have affected bacteria-phage coexistence. Most notably, population mixing increased the productivity of the environment (presumably through increased aeration), as shown by the greater bacterial densities in homogeneous microcosms in the absence of phage (Fig. 3b, densities averaged through time; t -test, $t = 6.93$, d.f. = 6, $P < 0.01$). Increased productivity may have destabilized coexistence in two ways. First, it may simply have sped up an inevitable increase in the proportion of resistant bacteria (and hence decline in phage densities) that would also have occurred in heterogeneous microcosm if left for longer. This however is highly unlikely, as the growth rate of bacteria is constrained to an average 100-fold increase every 24 h by the transfer regime. Second, more productive environments can theoretically ameliorate the cost of resistance by compensating for the associated trade-off with competitive ability (Hochberg & van Baalen, 1998), resulting in a lower proportion of sensitive cells in homogeneous microcosms. To test this we measured the growth rates of resistant bacteria relative to the sensitive ancestor in both environments. The greater productivity of the homogeneous environment had no significant effect on the cost of resistance in *P. aeruginosa*; resistant bacteria displayed similar relative growth rates in homogeneous and heterogeneous microcosms (growth rate of resistant bacteria relative to the ancestor under homogeneous ($w = 0.936 \pm 0.253$) and heterogeneous ($w = 0.921 \pm 0.013$) conditions not significantly different, t -test, $t = 1.47$, d.f. = 9, $P = 0.17$).

These results concur with findings from some predator-prey systems (Huffaker, 1958; Pimentel *et al.*, 1963; Luckinbill, 1973). In addition, these results consolidate observations from other bacteria-bacteriophage systems, which suggest that somewhat heterogeneous environments support stable coexistence [unshaken serially transferred microcosms (Buckling & Rainey, 2002); shaken serially transferred microcosms with permanent wall growth (Schrag & Mittler, 1996); mixed chemostats with wall growth (Chao *et al.*, 1977)] while in homogeneous environments, coexistence tends to be less stable [serially transferred microcosms without permanent wall growth (Schrag & Mittler, 1996; Lythgoe & Chao, 2003)]. However, in contrast to some other systems (Schrag & Mittler, 1996), coexistence between *P. aeruginosa* and PP7 was not reliant on permanent spatial refuges that persist for the duration of the experiment. In this study, any refuge from phage attack was ephemeral, lasting no longer than a single transfer, and arose through reduced dispersal in unshaken heterogeneous microcosms. These findings may be of importance to our understanding of biodiversity. For example, the disturbance of habitats such that natural

barriers to dispersal are removed is likely to destabilize coexistence between antagonistically interacting species. Furthermore processes that are likely to artificially increase population mixing in natural communities such as large-scale agriculture may also destabilize the coexistence of antagonistic species.

Acknowledgments

We thank Andrew Spiers and John Pannell for useful discussions, Iain Lamont for the kind gift of *P. aeruginosa*, Julie Stansfield for technical assistance and gratefully acknowledge funding from the Wellcome Trust.

References

- Bohannan, B.J.M. & Lenski, R.E. 2000. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.* **3**: 362–377.
- Bonsall, M.B., French, D.R. & Hassell, M.P. 2002. Metapopulation structures affect persistence of predator-prey interactions. *J. Anim. Ecol.* **71**: 1075–1084.
- Boots, M. & Sasaki, A. 1999. "Small worlds" and the evolution of virulence: infection occurs locally and at a distance. *Proc. R. Soc. Lond. B. Biol. Sci.* **266**: 1933–1938.
- Bowers, R.G., Boots, M. & Begon, M. 1994. Life-history trade-offs and the evolution of pathogen resistance: competition between host strains. *Proc. R. Soc. Lond. B. Biol. Sci.* **257**: 247–53.
- Brockhurst, M.A., Rainey, P.B. & Buckling, A. 2004. The effect of spatial heterogeneity and parasites on the evolution of host diversity. *Proc. R. Soc. Lond. B. Biol. Sci.* **271**: 107–111.
- Brockhurst, M.A., Buckling, A. & Rainey, P.B. 2005. The effect of a bacteriophage on diversification of the opportunistic bacterial pathogen, *Pseudomonas aeruginosa*. *Proc. R. Soc. Lond. B. Biol. Sci.* **272**: 1385–1391.
- Brockhurst, M.A., Morgan, A.D., Rainey, P.B. & Buckling, A. 2003. Population mixing accelerates coevolution. *Ecol. Lett.* **6**: 975–979.
- Buckling, A. & Rainey, P.B. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. Lond. B. Biol. Sci.* **269**: 931–936.
- Chao, L., Levin, B.R. & Stewart, F.M. 1977. A complex community in a simple habitat: an experimental study with bacteria and phage. *Ecology* **58**: 369–378.
- Ellner, S.P., McCauley, E., Kendall, B.E., Briggs, C.J., Hosseini, P.R., Wood, S.N., Janssen, A., Sabelis, M.W., Turchin, P., Nisbet, R.M. & Murdoch, W.W. 2001. Habitat structure and population persistence in an experimental community. *Nature* **412**: 538–543.
- Haraguchi, Y. & Sasaki, A. 2000. The evolution of parasite virulence and transmission rate in a spatially structured population. *J. Theor. Biol.* **203**: 85–96.
- Hilborn, R. 1975. The effect of spatial heterogeneity on the persistence of predator-prey interactions. *Theor. Popul. Biol.* **8**: 346–355.
- Hochberg, M.E. & van Baalen, M. 1998. Antagonistic coevolution over productivity gradients. *Am. Nat.* **152**: 620–634.
- Holyoak, M. 2000. Habitat patch arrangement and metapopulation persistence of predators and prey. *Am. Nat.* **156**: 378–389.

- Holyoak, M. & Lawler, S.P. 1996. Persistence of an extinction-prone predator-prey interaction through metapopulation dynamics. *Ecology* **77**: 1867–1879.
- Hosseini, P.R. 2003. How localized consumption stabilizes predator-prey systems with finite frequency of mixing. *Am. Nat.* **161**: 567–585.
- Huffaker, C.B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. *Hilgardia* **27**: 343–383.
- Lenski, R.E. 1991. Quantifying fitness and gene stability in microorganisms. *Biotechnology* **15**: 173–192.
- Lenski, R.E. & Levin, B.R. 1985. Constraints on the coevolution of bacteria and virulent phage: A model, some experiments, and predictions for natural communities. *Am. Nat.* **125**: 585–602.
- Luckinbill, L.S. 1973. Coexistence in laboratory populations of *Paramecium aurelia* and its predator *Didinium nasutum*. *Ecology* **54**: 1320–1327.
- Lythgoe, K.A. & Chao, L. 2003. Mechanisms of coexistence of a bacteria and bacteriophage in a spatially homogenous environment. *Ecol. Lett.* **6**: 326–334.
- Maynard Smith J. 1974. *Models in Ecology*. Cambridge University Press, Cambridge.
- Murdoch, W.W. 1977. Stabilizing effects of spatial heterogeneity in predator-prey systems. *Theor. Popul. Biol.* **11**: 252–273.
- Olsthoorn, R.C., Garde, G., Dayhuff, T., Atkins, J.F. & Van Duin, J. 1995. Nucleotide sequence of a single-stranded RNA phage from *Pseudomonas aeruginosa*: kinship to coliphages and conservation of regulatory RNA structures. *Virology* **206**: 611–625.
- Pimentel, D., Nagle, W.P. & Madden, J.L. 1963. Space-time structure of the environment and the survival of host parasite systems. *Am. Nat.* **97**: 141–167.
- Rainey, P.B. & Travisano, M. 1998. Adaptive radiation in a heterogeneous environment. *Nature* **394**: 69–72.
- Schrag, S.J. & Mittler, J.E. 1996. Host-parasite coexistence: the role of spatial refuges in stabilizing bacteria-phage interactions. *Am. Nat.* **148**: 348–377.
- Thompson, J.N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.* **153**: S1–S14.
- Thrall, P.H. & Burdon, J.J. 2002. Evolution of gene-for-gene systems in metapopulations: the effect of spatial scale of host and pathogen dispersal. *Plant Pathol.* **51**: 169–184.

Received 28 July 2005; accepted 27 August 2005