The Ecology and Evolution of Host-Parasite Interactions in Spatially Structured Populations

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by

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Abstract

Host-parasite coevolution, the reciprocal evolution of host defence and parasite counter-defence, has been implicated in driving a range of important ecological and evolutionary patterns. Populations of coevolving antagonists are often spatially subdivided into patches linked by dispersal, which is predicted to have consequences for the ecology, evolution and coevolution of interacting hosts and parasites. However, testing evolutionary hypotheses can be problematic in nature due to the large spatiotemporal scales typically involved. Populations of bacteria and their viral bacteriophage parasites can be a useful tool for studying coevolution, due to their ease of laboratory propagation and potential for rapid evolutionary dynamics. In this thesis I used an experimental evolution approach to test the effects of dispersal on the ecology and evolution of spatially structured coevolving populations of *Pseudomonas fluorescens* and phage Φ2. In chapter 2 I demonstrate that dispersal increased the spatial synchrony but decreased stability of bacterial population dynamics in the presence of phage, which drove deterministic multigenerational cycles that became phase locked through dispersal. In chapter 3 I demonstrate that intermediate rates of phage dispersal maximised both phage adaptation and the rate of coevolution, but that further increases in dispersal rate led to homogenization of subpopulations that impaired the rate of coevolution. In chapter 4 I demonstrate that the spatial pattern of dispersal affected the evolution of parasite local adaptation, specifically that unidirectional dispersal generated a geographic mosaic in phage adaptation leading to spatially structured phage local adaptation and maladaptation in the same metapopulation. In chapter 5 I demonstrate that demographic source populations had pacemaker effects on the rate of coevolution in metapopulations containing patches that varied in the strength of coevolutionary selection.

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Publications

The following published papers have arisen from this thesis, and are included as appendixes:

Vogwill T, Fenton A, Brockhurst MA. (2009): *Dispersal and natural enemies interact to drive spatial synchrony and decrease stability in patchy populations.* Ecology Letters (in press). (Appendix 1).

Vogwill T, Fenton A, Brockhurst MA. (2008): *The impact of parasite dispersal on antagonistic host-parasite coevolution.* Journal of Evolutionary Biology, 21(5): 824-9. (Appendix 2).

Vogwill T, Fenton A, Buckling A, Hochberg ME, Brockhurst MA. (2009): *Source populations act as coevolutionary pacemakers in experimental selection mosaics containing hotspots and coldspots*. American Naturalist, 173: E171-E176. (Appendix 3).

Chapter 1: General Introduction

1.1 Summary

As each chapter contains its own specific introduction, this chapter is used to introduce the ways of studying coevolution, the Geographic Mosaic Theory framework, and the use of bacteriophages as model parasites for testing coevolutionary hypotheses.

1.2 Coevolution

"It explains why cheetahs run fast, and why gazelles run fast too. It explains why the flowers of some orchids have extraordinarily long spurs to store their nectar, and why the moths that pollinate them have extraordinarily long tongues to drink it. It explains why we don't all succumb to diseases, and why diseases still exist."

David R Nash (2008)

Coevolution is reciprocal evolutionary change between two or more organisms, and it can be argued that the majority of evolutionary change is coevolutionary change (Thompson 2005). Antagonistic coevolution with parasites is inferred to be responsible for a wide range of biological and ecological phenomena (Thompson 2005), such as the evolution and maintenance of sex (Jaenike 1978; Hamilton 1980; Hamilton et al. 1990), and the evolution and maintenance of biodiversity (Haldane 1949; Thompson 2005). This is in addition to the obviously important impacts that hosts and parasites have on each other's ecological and evolutionary dynamics (Price 1980), such as their demography and population stability (Anderson and May 1978), and their respective levels of resistance, tolerance, infectivity and virulence (Bull 1994; Boots and Bowers 1999; Sasaki and Godfray 1999; Sasaki 2000).

Evidence of coevolution from natural populations can be detected by investigating either the spatial patterns of host-parasite interactions, or the temporal patterns of host-parasite interactions (Gaba and Ebert 2009). Temporal patterns of adaptation in host parasite systems can be detected using time-shift experiments, where the

fitness of hosts and parasites from multiple different time points are assessed against each other (Gaba and Ebert 2009). There are obvious practical barriers which prevent these types of experiments being commonly performed, as it may not be possible to store samples of both antagonists in stasis (Gaba and Ebert 2009). As a consequence, time-lagged selection on one antagonistic has been demonstrated several times, e.g. (Dybdahl and Lively 1998; Fenner and Fantini 1999), but examples of reciprocal time-lagged selection in a natural environments is rarer. However, Decaestecker et al (2007) utilised the dormant stages of the water-flea, *Daphnia magna* and its bacterial parasite, *Pasteuria ramosa,* which had been naturally archived in pond sediment to demonstrate coevolution using a time-shift approach. They reported that *P. ramosa* infectivity against contemporary hosts was greater for contemporary parasites, than for either parasites from the past or future, which is indicative of time-lagged frequency dependent coevolution where the parasite population tracks the locally common host genotype.

The spatial patterns of host-parasite interactions can be used to infer coevolution, and are often assessed in terms of local adaptation. Local adaptation is defined as higher mean fitness in sympatric environments rather than allopatric ones, with local maladaptation being the reverse (Kawecki and Ebert 2004). For example, if a parasite population is locally adapted to its host, it implies that host resistance has diverged between host populations, and that the local parasite population has evolved to overcome that resistance (Price 1980). Numerous factors are implicated by theoretical or empirical studies in affecting the sign and magnitude of local adaptation (reviewed in (Greischar and Koskella 2007; Hoeksema and Forde 2008)). In particular, the relative amounts of dispersal of hosts and parasites is predicted to be a major determinant of local adaptation, with whichever antagonist is more dispersive being more likely to show local adaptation (Greischar and Koskella 2007; Hoeksema and Forde 2008). However, as geographic studies of coevolution only take snapshot data of the current quantities and distributions of traits (Laine 2009), they do not provide direct evidence of coevolution in natural populations (Gaba and Ebert 2009). However these studies do reveal that coevolutionary interactions not only show pronounced geographic

structuring, but that this geographic structuring also has a major impact on the rate and direction of coevolution.

1.3 The Geographic Mosaic of Coevolution

In an attempt to incorporate both the rapid temporal and the broad spatial dynamics of coevolution, John N. Thompson has proposed The Geographic Mosaic Theory of Coevolution (GMTC) as a conceptual framework in which study coevolution (Thompson 2005). Essentially, it is not highlighting the trivial fact that interspecific interactions do show geographic variation, but it is suggesting without spatial variation in biotic and abiotic factors, coevolution would be slow, rare or even absent (Nash 2008). In this framework, the three fundamental processes that drive coevolutionary interactions are referred to as: geographic selection mosaics; hot spots and cold spots of coevolution; and trait remixing (Thompson 2005). In other words, natural selection on interspecific interactions must vary between populations in its direction or ultimate outcome, such as by a genotype by genotype by environment interactions; where reciprocal selection does occur it must vary in its intensity or presence; and the genetic structure of these coevolving populations must be continually changing by dispersal, gene flow, mutation or extinction.

These three processes are predicted to result in three patterns: spatial variation in coevolved traits; mismatched levels of coevolved traits within some coevolving populations; and few species level coevolved traits (Thompson 2005). However, these patterns can result from many different processes and the existence of them is the reason why the GMTC was developed, and cannot be used as its proof as well (Gomulkiewicz et al. 2007). Based on the stringent criteria outlined by Gomulkiewicz et al (2007) no system has actually demonstrated that a complete geographic mosaics of coevolution as envision by Thompson (2005) is actually occurring in natural populations (Nash 2008).

However, there is growing evidence for the individual processes of the GMTC occurring in natural populations. Geographic variation in the strength of coevolution has been inferred in numerous natural populations (Benkman 1999;

Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thompson and Cunningham 2002; Thrall and Burdon 2003; Thompson 2005; Laine 2006; Toju and Sota 2006; Hanifin et al. 2008). In some cases, this variation in the strength of coevolution is suggested to be associated with an underlying selection mosaic. In a recent review, Laine (2009) highlighted that these spatially variable selection pressures are inferred to be caused by a wide range of abiotic conditions, such as latitude (Hoeksema and Thompson 2007) or temperature (Toju 2008), as well as biotic conditions, such as the presence of other hosts (Antonovics et al. 2002) or the presence of competitors (Parchman and Benkman 2008). Trait remixing has been described as the 'uncharted waters' of coevolution (Thompson 2005), as although the processes responsible for trait remixing are fundamental to population genetics, they have rarely been empirically assessed in relation to selection mosaics or hotspots and coldspots of coevolution (Gomulkiewicz et al. 2007).

1.4 Testing Evolutionary Hypothesises: Microorganisms in Microcosms

"We see nothing of these slow changes in progress, until the hand of time has marked the long lapses of ages, and then so imperfect is our view into the long past geological ages that we only see that the forms of life are now different from what they were."

Charles Darwin (1859)

If coevolution does occur over the predicted wide geographic ranges, it follows that investigation also need to be on these large and unpractical scales. Coevolutionary changes, as with evolutionary changes in general, can also occur over slow temporary scales and present challenges in its observation and experimental manipulation. As a consequence, although coevolution is often inferred to be involved in natural populations, it is very rarely explicitly demonstrated to be occurring (Buckling and Rainey 2002; Brockhurst et al. 2007b). Microbial experimental systems can provide a convenient solution to this problem (Buckling et al. 2009); they can be grown in controlled, replicate microcosms under a diverse range of conditions, as well as enabling the comparison of genotypes between spatially and temporally separated populations.

Microbes and their associated parasites and predators therefore allow aspects of coevolution to be studied which cannot be readily observed in natural populations (Bohannan and Lenski 2000; Brockhurst et al. 2007b).

There are many obvious advantages to using microbes as experimental models in evolutionary biology, as well as obvious drawbacks, which are discussed in a number of recent reviews (Jessup 2004; Brockhurst et al. 2007b; Jessup and Forde 2008; Bennett and Hughes 2009; Buckling et al. 2009). Microbes, however, are more than just convenient experimental tools. Indeed, doing something just because it is experimentally convenient is rarely sufficient justification; justification requires that a system must also posses some biological relevance as well (Krogh 1929). It is here argued that microbial experimental evolution can be more a mere experimental tool because microbes are far more important, diverse and complex than often assumed. They drive the earth's geochemical cycles, are major regulators of population densities and dynamics including humans and their crops and livestock, and are major selection pressures on the life-history of all metazoans as well as other microbes. While their sheer numerousness and diversity dwarfs those of other organisms by several orders of magnitude (Odonnell et al. 1994; Torsvik et al. 2002; Horner-Devine et al. 2004), they also have more complex ecologies and biologies than is often assumed: they age, cooperate, cheat, parasitize, predate, and compete (Buckling et al. 2009).

1.5 Bacteriophages as model Parasites

Numerous microbial experimental systems are developed to study differing aspects of evolutionary biology. For host-parasite coevolution, the viral bacteriophage parasites of bacteria have been used to study aspects of coevolution for over sixty years. The first inference of the existence of prokaryotic viruses was by Earnest Hankin in 1896 when he noted the bactericidal action of the water of the Jumna and the Ganges against cholera (Hankin 1896). In 1915, Frederick Twort reported on '*the nature of ultramicroscopic viruses*' (Twort 1915) and theie properties as bacteriolytic agents, while independently in 1917 Felix d'Herelle coined the term bacteriophages to describe the viral parasites of prokaryotes

(D'Herelle 1917). Initial estimates suggested phages were ubiquitously distributed across all environments globally, but of relatively low densities in all, and were consequently considered of little importance (Marsh and Wellington 1994). Bacteriophages have since been shown to be the most numerous clade of organisms on the planet, with approximately 10^{31} phage particles in the world, ten-fold more numerous than their bacterial hosts (Whitman et al. 1998; Suttle 2005; Hatfull 2008). They are now believed to be the major determinants of bacterial density and diversity and are therefore highly significant for both bacteria and consequently anything affected by bacteria (Marsh and Wellington 1994).

Bacteriophages physically consist of genetic material surrounding by a protein capsid. The protein capsid can take many forms and phages can possess a diverse range of morphologies (Weinbauer 2004), but it is estimated that 96% of phages possess the stereotypical tails and icosohedral heads (figure 1a). Genetically, phages vary from the tiny RNA and DNA phages with genomes smaller than 4kbp (Calender 1988), to the 'jumbo' DNA phages with genomes of over 200kbp (Hendrix 2009). The majority of phage open reading frames have no matches in genetic databases (Comeau et al. 2008), and in contrast to cellular 'living' organisms, phages do not possess universal genes, such as the 16s ribosomal sub-unit, that would allow the construction of a global phylogeny (Hendrix et al. 1999). Instead, phylogenies of groups of phages have been constructed based on genome architectures – the order and arrangement of genes within a genome (Comeau et al. 2007; Hatfull 2008). Analysis of this has revealed that phage genomes show high levels of mosaicism. Essentially, phage genomes are made up of modules of genes which can be interchanged via horizontal gene transfer between different phage populations (Hendrix et al. 1999). Although mosaicism and horizontal transfer is *a* feature of bacterial genomes, it is *the* feature of phage genomics, and an individual phage's genome is suggested to all be drawn from a common global gene pool, accessible to all phage population to at least some extent (Hendrix et al. 1999).

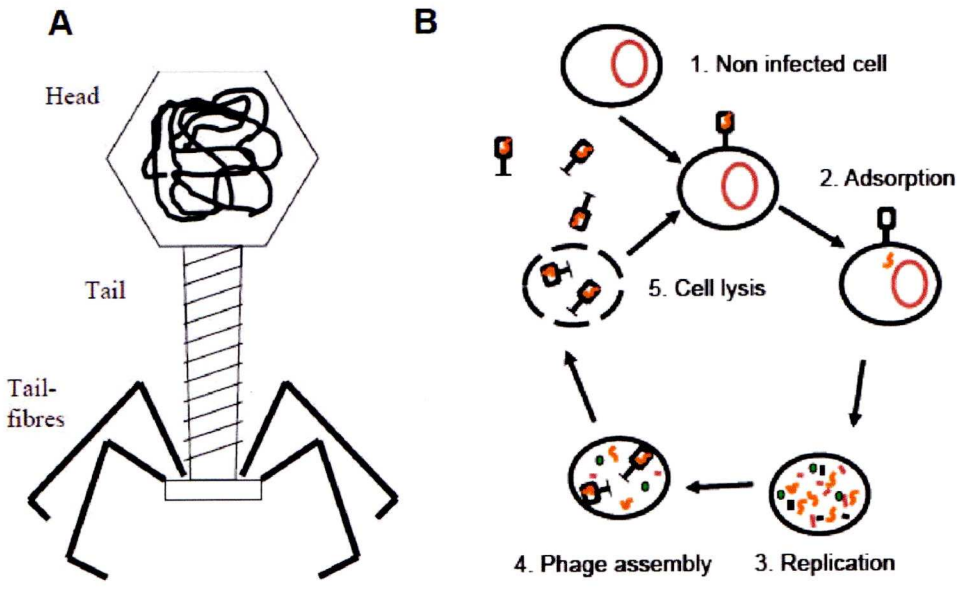

Figure 1.1: Bacteriophage characteristics: (A) the morphology of a tailed bacteriophage in the order Caudovirales; (B) the life-cycle of a lytic virus (courtsey of V. Poullain).

1.5.1 A Life-history for the non-living

Phages are obligate viral parasites of prokaryotes. They reproduce by injecting their DNA into a host cell and then using the host's to produce new viral particles, before ultimately lysing the host cell to release these new virons. Phage life-history can be divided into three distinct phases; the absorption period, the eclipse period, and the latent period (figure 1b;(Weinbauer 2004)). The absorption phase of a phages life-cycle has two stages. The first of these involves the reversible binding to a receptor site on the bacterial outer envelope membrane (OM), after which the phage particle drifts across the surface of the OM until it locates a receptor for the second, irreversible stage of binding (Lenski 1988a; Weinbauer 2004). This is followed by the eclipse period, which is the time between binding to a host cell and the first appearance of intracellular mature virons. After irreversible binding, phage particles then inject their genetic material into the host, and may immediately begin to replicate. However some phages, referred to as lysogenic or temperate phages, do not immediately begin replication upon entering a host and instead integrate themselves into their host's genome and extend the eclipse period (Lenski 1988a). Phages in this state are referred to as

prophages and enable the phage to be vertically transmitted to daughter cells. The decision of when to end this 'dormant' stage of the lysogenic cycle is thought to depend on host physiology (Chibani-Chennoufi et al. 2004): a fast growing host-cell with abundant resources will produce a greater number of phage particles, as well as being more likely to be surrounded by similarly nutritionally well-endowed con-specifics.

The latent period of a phage is the time between the emergence of mature virons and their release via lysing of the host cell. The length of this latent period is positively correlated to the burst size of a phage - the number of phage particles released at host lysis. A trade-off therefore exists between the number of particles produced at lysis and the speed with which they are able to infect a new host, and is often compared to the relationship between virulence and transmission in parasite ecology (Bull 2006). Host density is predicted to be a key determinate of the optimal relationship between the two: high host density should select for short latent times and low burst sizes, while low density is predicted to select for the opposite (Bull 2006).

Host density is also considered a major determinant in another trade-off in phage life-history, between the breadth of host range and the absorption affinity to any particular host (Duffy et al. 2006). Increasing host-range has been shown to be reduce binding affinity to a particular host by antagonistically pleiotropy (Duffy et al. 2006). Breadth of host range is predicted to depend on both overall bacterial density as well as the relative quality of these hosts (Guyader and Burch 2008; Heineman et al. 2008). Due to the low density of individual bacterial species but high diversity of species in bacteria populations, phages are predicted to be under selection to be polyvalent and be able to infect a wide variety of bacterial strains (Chibani-Chennoufi et al. 2004).

Despite being obligate parasites, phages must still also interact and survive within their abiotic environment (De Paepe and Taddei 2006). Environmental levels of salt, temperature and UV are the main sources of phage 'death' – inactivation of a phage particle by capsid damage or rupturing (De Paepe and Taddei 2006). Capsid stability is positively correlated with capsid molecular mass, but

negatively correlated with density of DNA packed within it (De Paepe and Taddei 2006). Interestingly, capsid mass is negatively correlated with phage multiplication rate within a host, and provides a mechanistic link between survival and reproduction rate that is central to much of ecological life-history theory (De Paepe and Taddei 2006).

1.5.2 Should phages be lysogenic and polyvalent?

Natural communities of prokaryotes are predicted to consist of a high diversity of bacterial species, but any individual strain is at a relatively low density (Bruttin et al. 1997; Wommack and Colwell 2000; Chibani-Chennoufi et al. 2004). When combined with the inferred low productivity of natural environments, phages are predicted to be predominantly lysogenic and highly polyvalent (Chibani-Chennoufi et al. 2004). However, only 10% of isolated phages are lysogenic and the majority of phages show very strong strain specificities (Chibani-Chennoufi et al. 2004),. There is evidence that the prevalence of both lysogeny and polyvalency increases in more oligotrophic environments (Hennes et al. 1995; Long et al. 2008; Williamson et al. 2008), as well as evidence that bacteria harbour more prophages in winter rather than the more productive summer (McDaniel et al. 2002). However the dominance of lytic phages with narrow host ranges suggests that current models do not adequately describe phage ecology (Chibani-Chennoufi et al. 2004), and that there may be other physiological or ecological barriers preventing the evolution of broad host-ranges or lysogenic life-histories (Guyader and Burch 2008).

1.5.3 Bacterial Defences against Phages

1.*5.3.1 Preventing absorption*

Bacteria have evolved a range of defences against phages which target different aspects of the phage life-cycle. The most commonly observed evolution of defence under laboratory conditions is the emergence of bacterial mutants with

altered receptor sites to which phages cannot bind, which therefore prevents either of the stages of absorption (Lenski 1988a). Alternatively, bacteria can prevent absorption by stopping phages from locating a receptor site by either over-expressing exopolysaccharides which mask these initial interaction sites, or similarly forming biofilms and restricting extra-cellular phage movement (Forde and Fitzgerald 1999; Sutherland 2001; Weinbauer 2004).

1.5.3.2 Preventing replication

Restriction-modification (RM) systems are mobile genetic elements which protect bacteria by digesting phage and other foreign DNA, and are found in about one quarter of bacteria (Wilson 1991; Wilson and Murray 1991). They operate via restriction endonucleases which cleave specific DNA sequences, while cognate methylates prevent self restriction by adding methyl groups to bacterial DNA (Wilson and Murray 1991). Interestingly, RM-systems are no longer considered symbiotic to their hosts and instead are a form of intracellular parasite, which although does protect the host against other mobile genetic elements, it only does so for its own benefit (Kobayashi 2001). Similar to RM-systems are clustered regularly interspaced short palindromic repeats (CRISPR), which provide acquired immunity against phages via RNAi's digesting foreign DNA, and are found in the majority of prokaryotic genomes (Barrangou et al. 2007). Specifically, CRISPR units contain stretches of phage DNA (called spacers) which are used to target RNA interference silencing complexes, which bind to and cleave sequences complimentary to the spacer (Barrangou et al. 2007). The last line of defence against phages is referred to abortive infection (Weinbauer 2004), which is effectively a form of programmed cell death designed to prevent the release of mature phage virons (Forde and Fitzgerald 1999).

1.5.4 Bacteria-Phage Coevolution in Natural Populations

The natural ecology of bacteriophages, as with all microbes, is in its infancy (Ash et al. 2008). However, studies of natural bacteria-phage populations utilising novel molecular techniques are beginning to unravel the workings of coevolution

in natural communities (Heidelberg et al. 2009; Wilmes et al. 2009). Analysis of CRISPR sequences from environmental bacterial samples has revealed that they are amongst the most rapidly evolving parts of bacterial genomes (Tyson and Banfield 2008; Heidelberg et al. 2009), as well as revealing that bacteria-phage coevolution is both rapid and geographically structured. Tyson and Banfield (2008) found that CRISPR loci were highly polymorphic in otherwise nearly clonal bacterial populations. Similarly, Andersson and Banfield (2008) showed that although CRISPR sequences were rapidly evolving, only the most recently acquired spacer sequences matched the majority of currently active phages, as phage populations showed evidence of extensive homologous recombination to evade host defences. Moreover, Kunin et al (2008) showed that the CRISPR units of bioreactor bacteria show pronounced geographic structuring, despite the rest of the bacteria's genome showing no evidence of geographic structuring. Similarly, Silander et al (2005) found no evidence of structuring in *Cystoviridae* phage genome modules across the whole of North America, except for the module which carries the genes responsible for host specificity.

Using more traditional microbial techniques, Vos et al (2009) found phages are locally adapted to their bacterial hosts on a scale of centimetres in soil. Conversely, Waterbury and Valois (1993) showed that cyanobacteria are resistant to the majority of their co-occurring phages in both inshore and offshore seawater, but susceptible to phages from other samples. This contrast suggests structuring and the extent of population mixing in an environment have an important role in bacteria-phage evolution. Moreover, phage dispersal between environments is also thought to be important. When bacterial strains are released into an environment which are resistant to the phages currently located there, novel immigrant phages which can infect these new host strains quickly appear (Bruttin et al. 1997). Furthermore, viral movement between environments was indicated to be having a greater impact on the genetic diversity of phage populations than mutation was in hot spring communities (Snyder et al. 2007). This suggests that although phages due evolve rapidly in natural environments, dispersal also has an important role in phage ecology.

1.5.5 Coevolution in the Laboratory

Although comparatively little is known about the temporal and evolutionary dynamics of bacteria and phages the field, the behaviour, genetics and evolution of phages in laboratories are particularly well documented (Bohannan and Lenski 2000; Brockhurst et al. 2007b; Buckling et al. 2009). As a laboratory organism, *E. coli* has few equals in terms of the diversity of strains and genetic resources that are available. Many landmarks in biology were achieved using *E. coli* and its phages, such as the confirmation of DNA as the unit of inheritance (Hershey and Chase 1952), the discovery of mRNA (Volkin and Astrachan 1956), the first full genome sequence (Sanger et al. 1977), and that selection works on standing genetic diversity rather than inducing a change in it (Lederberg and Lederberg 1952). The consequence of the latter of these is that bacteria resistant to phages are already present in cultures prior to exposure to bacteriophages, and when phages are introduced bacterial populations quickly undergo a selective sweep resulting in resistant bacteria becoming dominant (Lenski 1988a). If there is no cost associated with this resistance under laboratory conditions, as in the case of phage T5, both susceptible bacteria and the phage are driven extinct (Lenski and Levin 1985). However, in most cases these mutations are costly and stable coexistences between phages, susceptible bacteria and resistant bacteria occur due to trade-offs between resistance and growth rate (Lenski 1988a). However, the density of resistant bacteria is generally several orders of magnitude greater than the density of the susceptible bacteria; moreover, the density of resistant bacteria does not typically fluctuate to a great extent through time and consequently neither does the overall density of bacteria (Yoshida et al. 2007). However, this masks the 'cryptic' dynamics between phages and susceptible bacteria, which undergo predator-prey cycles due to the tight ecological coupling between the two populations (Yoshida et al. 2007).

Within individual microcosms, multiple different resistant mutations are observed (Lenski 1988a). In most *E. coli*-phage interactions all mutations uniformly confer complete resistance to phages, but the metabolic costs associated with them is variable between individual mutants, as well as varying by genetic background and environment (Lenski 1988a). For example, less than expected costs were

associated with mutants evolved to be resistant to both T4 and lambda phage when compared to resistance to either phage in isolation (Bohannan et al. 1999). However, single mutations which are more likely to confer cross-resistance to other phages have been shown to confer greater costs (Lenski 1988b). In addition, compensatory selection has been shown to occur under longer term selection experiments that reduce the size of this cost over-time (Lenski 1988b). Genotype by environment interactions have also been shown to influence the magnitude of these costs including environmental productivity (Forde et al. 2008), carbon source (Bohannan et al. 1999) and temperature (Bohannan and Lenski 2000).

1.6 Host-Parasite Coevolution: Pseudomonas fluorescens SBW25 and SBW25Φ2

Despite the rapid evolution of host resistance, phages with increased host ranges are relatively rare for *E. coli* phages. The notable exception is phage T7, although even this only shows a one-step increase in host-range, and consequently only a two-stage increase in bacterial resistance (Chao et al. 1977). This asymmetry in evolutionary potential led to the questioning of whether long term escalatory arms-races actually occurred in bacteria-phage systems (Lenski 1984; Lenski and Levin 1985). However, this is now believed to be an artefact of both the domestication of *E. coli* and its phages, and an increasing number of systems have revealed phages which undergo repeated increases in host range (Brockhurst et al. 2007b). The best characterised phage-host arms race is between *Pseudomonas fluorescens* SBW25 and its lytic bacteriophage parasite SBW25Φ2, and is the system utilised in this thesis.

1.6.1 Pseudomonas fluorescens SBW25

Pseudomonas fluorescens is gram-negative gamma proteobacteria. Its natural ecology is varied and it has been described as a common marine bacterium (Poirier et al. 2008), a common soil bacterium (Silby et al. 2009), a common spoilage organism (Arakawa et al. 2009), a clinical pathogen (Picot et al. 2001; Rossignol et al. 2009), an aquaculture pathogen (Zhang et al. 2009), a biocontrol bacteria (Couillerot et al. 2009), as well as both plant pathogenic (Saygili et al.

2004) and plant symbiotic bacterium (Albareda et al. 2006). Essentially, *P. fluorescens* is not a fussy organism and has a diverse and flexible metabolism. Although rarely of medical importance, it was recently responsible for infecting 80 cancer patients via contaminated saline flushes in the United States between 2004 and 2006 (Gershman et al. 2008). Economically, it is responsible for soft rot of fruit and vegetables (Cui and Harling 2006) as well as the spoilage of milk (Werner and Hotchkiss 2006). However not all environmental isolates possess the necessary enzymes for plant cuticle decomposition (Dogan and Boor 2003), and only some eco-types of *P. fluorescens* are capable of causing soft rot, milk spoilage or being opportunistic pathogens of plants (Dogan and Boor 2003). Furthermore, some isolates are considered plant symbionts and are studied as potential bio-control organisms, and have been shown to be capable of protecting plant roots from attack by pathogenic fungi and nematodes (Couillerot et al. 2009). The experimental strain SBW25 was originally isolated from a sugar beet rhizosphere at Wytham farm at Oxford University (Rainey and Bailey 1996), and is a plant-growth promoting organism capable of colonising both plant roots and leaves.

Under standard laboratory conditions of 28°C in liquid King's B media it undergoes 3-4 generations a day, and is normally cultured by serial transfer every two days. Under these conditions it will reach stationary phase at 10^9 colony forming units per ml. The initial interest in SBW25 as a model system stemmed from its rapid morphological diversification: an adaptive radiation in response to intra-specific competition (Rainey and Travisano 1998). Microbial growth in static liquid media results in a vertical oxygen gradient. Ancestral cells of SBW25 are primarily aerobic and planktonic, and hence adapted to the liquid broth phase of the media and consequently under selection for increased resistance to anaerobic stress at the base of the tube as well as selection to maintain position at the oxygen-rich air-broth interface. The latter of these is achieved by increasing extra-cellular cellulose excretion and creating a biofilm across the surface of the tube (Bantinaki et al. 2007). These biofilms are cooperative structures have been used to study factors affecting the evolution of cooperation and cheating (Rainey and Rainey 2003).

1.6.2 Coevolution with SBW25Φ2

SBW25Φ2 is a podoviridae with linear double-stranded 40kb genome (Morgan et al. 2007), and is a member of the *E. coli T7-like* genus, members of which can infect a wide variety of gamma proteobacteria (Ceyssens et al. 2006). The ancestral lytic cycle takes about 30-40 minutes to complete and leads to the release of approximately 40 viral particles (V Poullain, personal communication). Host resistance occurs via modifications to the currently unknown receptor sites (Buckling and Rainey 2002), which are countered by phage host-range mutants that have reductions in the length of its tail fibre gene, as well as diversification of another two genes of unknown function (Paterson et al (in submission)). Selection in this arms-race has been shown to be largely as a result of directional selection (Buckling and Rainey 2002), and most phage host range mutants are still able to grow on ancestral bacteria, although phage mutants do occur that lose this ability (Poullain et al. 2008; Benmayor et al. 2009). This arms race can continue for 60-70 experimental transfers (120 - 140 days) with persistent increases in both phage infectivity and bacterial resistance (Buckling and Rainey 2002). After this period, cyclic frequency-dependent selection becomes the central driving force of within population selection (Morgan et al. 2005), although the specific mechanism of this remains unclear. These increases in bacterial resistance have been shown to be costly to bacteria (Brockhurst et al. 2004), and this cost escalates with increasing extent of resistance range (Buckling et al. 2006). Phages also suffer from a reduction in growth rate on ancestral bacteria (Buckling and Rainey 2002; Poullain et al. 2008), which is likely as a result of antagonistic pleiotropy, but also being as a result of deleterious mutation cannot be ruled out.

1.6.3 The Importance of Dispersal and Population Mixing

Within an individual population, SBW25 has a higher evolutionary potential than SBW25Φ2, and typically the majority of bacteria are resistant to their contemporary, sympatric phages, except when a new phage host-range mutant evolves (Buckling and Rainey 2002). However, a bacteria with an enhanced resistance range quickly evolves to restore the pattern of bacterial dominance.

This pattern is thought to be due to the bacteria having a population size several orders of magnitude larger than their parasites (10^9 per ml per opposed to 10^6 per ml), and therefore likely to possess a greater within population level of genetic diversity (Buckling and Rainey 2002). Phages show no evidence of local adaptation or local maladaptation during the early stages of coevolution due to the rapid directional selection, but become locally maladapted when selection becomes increasingly cyclic (Buckling and Rainey 2002; Morgan et al. 2005).

In contrast, when between tube dispersal is introduced both these patterns are reversed (Brockhurst et al. 2007b). When bacteria and phages are migrated at equal rates between tubes, phages benefit far more than their hosts, and levels of infectivity become generally high and resistance levels low (Morgan et al. 2007). This is thought to be due to diversity being a limiting factor for within-tube phage adaptation, which is augmented by migration. In contrast, bacterial adaptation is limited by the strength of selection imposed by the less adaptable phages (Buckling and Rainey 2002; Morgan et al. 2007). Moreover, phage dispersal reverses the within tube pattern of local maladaptation and phages become locally adapted (Morgan et al. 2005). Conversely, bacterial dispersal has no significant effect of phage local adaptation as again bacterial diversity is not a limiting factor (Morgan et al. 2005). This confirms the importance of the reciprocal nature of coevolution, and that during arms-races the strength of selection and the evolutionary potential of both antagonists interact to drive coevolution.

Within-tube mixing has also been shown to alter the balance of coevolution (Brockhurst et al. 2003). Despite a standard tube only containing six ml of media, relatively large amounts of within tube heterogeneity still occur, and bacteria populations contain a variety of specialist and generalist phenotypes with varying resistant ranges (Poullain et al. 2008). When cultures are grown in static incubators, levels of within-population mixing are low enough to allow localized bacteria-phage arms-races to occur in different parts of the same population, as well as allowing temporary spatial refuges from phages (Schrag and Mittler 1996; Brockhurst et al. 2003) . However, cultures grown with intermittent shaking prevents this differentiation and bacteria are more likely to be exposed to an infectious phage (Brockhurst et al. 2003). Furthermore, phages experience an

increase in population density due to the increase access to susceptible hosts. As a consequence, bacteria are under a stronger selection for resistance, and consequently phages are under stronger selection for infectivity, and the overall rate of coevolution increases (Brockhurst et al. 2003).

Other ecological processes have also been shown to alter coevolutionary outcomes in this system. Environmental productivity has been shown to correlate with the rate of increase in resistance, infectivity and the overall rate of coevolution, via increases in both the population densities of bacteria and phage, as well as reductions in the relative costs of resistant mutations by environment by genotype interactions (Lopez-Pascua and Buckling 2008). The rate of increase in phage host-range has also been shown to be dependent on the ratio of resistant to susceptible hosts (Benmayor et al. 2009). When resistant hosts are too dominant within a tube, phage populations are likely to drive any susceptible hosts extinct prior to the emergence of a host-range mutant (Benmayor et al. 2009). Conversely, excessive numbers of susceptible hosts limits the selective advantage of an increased host range (Benmayor et al. 2009), and instead selection favours individuals with increased growth rates on the ancestral host (Poullain et al. 2008).

1.7 Thesis Structure

This thesis extends pervious work to examine how the different ways of introducing spatial structuring can influence the dynamics of the interaction between SBW25 and SBW25Φ2. There are multiple different ways of introducing structuring to laboratory populations of microbes, each of which captures of different aspect of the spatial structure of natural populations. The consequences of the alteration of differing aspects of structuring will impact upon on host-parasite interactions in a variety of ways. Here, I focus on how spatial structuring affects the rate and direction of coevolution, as well as the ecological dynamics during host-parasite coevolution.

Chapter 2 examines how dispersal in spatially structured populations affects the stability and synchrony of host population dynamics.

Chapter 3 examines how the rate of parasite dispersal in spatially structured populations affects the dynamics and outcomes of coevolution.

Chapter 4 examines how the spatial dispersal network (i.e., the topology of patches and pattern of dispersal) alters the coevolutionary process, focussing on the evolution of parasite local adaptation.

Chapter 5 examines how dispersal between coevolutionary hotspots and coldspots affects coevolutionary dynamics.

Chapter 2: Dispersal and natural enemies interact to drive spatial synchrony and decrease stability in patchy populations

2.1 Abstract

Spatial synchrony is widespread in natural populations but the mechanisms that underpin it are not yet fully understood. Two key biotic drivers of spatial synchrony have been identified: dispersal and trophic interactions (e.g., natural enemies). We used spatially structured, patchy bacterial populations to show that although increased dispersal always enhanced spatial synchrony of fluctuations in bacterial abundance, this effect was far stronger in the presence of a bacteriophage parasite. Bacteriophages drove strong within patch fluctuations in bacterial abundance that became phase locked through dispersal. Furthermore, the way in which stability, measured as constancy, responded to increasing dispersal was qualitatively different depending on whether parasites were present or not. Patch-level constancy decreased with dispersal in the presence of parasites, whereas dispersal increased patch-level constancy in the absence of parasites. Population-level constancy also decreased with dispersal in the presence of parasites, but was unaffected by dispersal in the absence of parasites. These contrasting patterns were likely due to the different role played by dispersal in the presence and absence of parasites, synchronizing dynamics in the former case and averaging stochastic fluctuations in the latter. Taken together, our findings suggest that dispersal and natural enemies can interact to drive spatially synchronous population fluctuations that decrease stability at both the patch and population-level.

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2.2 Introduction

Spatial synchrony of ecological dynamics is pervasive in natural populations (reviewed in Bjornstad et al. 1999; Liebhold et al. 2004). Dispersal and trophic interactions have emerged as key biotic determinants of spatial synchrony (Liebhold et al. 2004). However, the precise way in which each factor affects synchrony, and the consequences for population stability and persistence remain the focus of considerable research (Blasius et al. 1999; Holland and Hastings 2008). A robust prediction of a range of theoretical models is that dispersal between patches can synchronize fluctuations that arise from similar driving processes (Bjornstad et al. 1999; Liebhold et al. 2004). Indeed, comparisons of species that differ in dispersal ability suggest that more dispersive species often display more spatial synchrony (Paradis et al. 1999). However, this is not always the case and spatial synchrony may instead be more strongly influenced by climactic factors (Peltonen et al. 2002), or may depend on the spatial scale at which synchrony is measured (Sutcliffe et al. 1996). Trophic interactions, particularly those with natural enemies, have long been thought to drive population fluctuations (Hanski et al. 1993; Krebs et al. 1995; Hudson et al. 1998). Theory suggests that interaction with a spatially synchronized natural enemy can drive spatial synchrony of the exploited species (Ims and Steen 1990; de Roos et al. 1998). Such processes are thought to underpin distribution and abundance patterns in a number of natural systems (Small et al. 1993; Ims and Andreassen 2000).

Dispersal and natural enemies are also likely to affect population stability. In this paper we focus on the constancy component of stability, which measures the tendency for abundance to remain unchanged through time (Grimm and Wissel 1997). Low constancy implies temporally fluctuating abundance, while high constancy implies temporally constant abundance. Constancy can be measured at the level of an individual patch, giving an estimate of abundance fluctuations at a local scale, or at the whole population level, giving an estimate of abundance fluctuations at a regional scale (Dey and Joshi 2006). Whether patch and

population measures of constancy are correlated depends upon the degree of spatial synchrony exhibited by the population.

The effect of dispersal on population stability has been modelled extensively. In general, a non-linear hump-shaped relationship between dispersal rate and population stability is predicted by both single-species (Gyllenberg et al. 1993; Hastings 1993) and victim-enemy models (Reeve 1988; Taylor 1990). This arises because, in patchy populations where patch abundances fluctuate asynchronously, recolonization may be insufficient at very low levels of dispersal to counter-balance extinctions of declining patches leading to low population stability. Moderate increases in dispersal may improve population stability because dispersal between out-of-phase patches allows recolonization of declining patches. However, further increases in dispersal are likely to synchronize abundance fluctuations across patches, preventing rescue effects (Brown and Kodric-Brown 1977) and potentially decreasing population stability (Heino et al. 1997). Some models predict that the effects of dispersal on population stability are likely to be stronger in the presence of natural enemies (Rohani et al. 1996).

Clearly, the effects of dispersal and natural enemies on local and regional dynamics have the potential to interact, resulting in counter-intuitive outcomes on ecological dynamics. A number of studies have tested the effects of dispersal on synchrony and / or stability in spatially structured single-species (Lecomte et al. 2004; Dey and Joshi 2006) or victim-enemy populations (Holyoak and Lawler 1996; Holyoak 2000; Ellner et al. 2001; Bonsall et al. 2002). However, few experimental studies to our knowledge have directly compared effects of dispersal on spatial synchrony and stability in the presence and absence of a natural enemy. There are several likely reasons for this, which include the large spatial and temporal scales involved in studies of natural systems. In addition there are difficulties associated with excluding natural enemies, accurately measuring and manipulating dispersal rates, and controlling for extrinsic variables in nature. However, such difficulties can be overcome by using laboratory populations of fast replicating microbes (Jessup 2004; Buckling et al. 2009). We propagated replicate 64-patch spatially structured populations of *P. fluorescens* SBW25 with and without bacteriophage SBW25Φ2, under 3 scales of dispersal (global,

localized and none). We explored how dispersal and natural enemies interact to affect spatial synchrony of ecological dynamics and population stability.

2.3 Materials and Methods

2.3.1 Culturing Techniques

Each population was propagated in 64 wells on a 96-well microtitre plate (i.e., an 8-well by 8-well grid), each well containing 100µl of King's B liquid media. 18 replicate phage-free spatially structured populations were initiated with approximately 1.7×10^6 bacterial cells per well and 18 replicate phage containing populations were initiated with 1.7×10^6 bacterial cells and 170 viral particles per well using a 96-pin replicator. These densities were chosen to be equivalent to the starting population densities previously used in experiments with this system (Buckling and Rainey 2002). Populations were propagated by serial transfer for 12 transfers (every two-days 1µl of each well was used to inoculate a fresh well using a 96-pin replicator) under one of the following dispersal regimes: global – all patches were pooled and homogenized prior to transfer; localized – the contents of all eight wells in each row were pooled and homogenized prior to even-numbered transfers, the contents of all eight wells in each column were pooled and homogenized prior to odd numbered-transfers; none – no between well mixing occurred prior to transfer.

2.3.2 Analysing Synchrony and Constancy

After each growth cycle, we measured bacterial abundance in each well as absorbance at 630nm using an optical density plate-reader (Biotek EL800). To estimate spatial synchrony we calculated cross correlations at lag-zero of the first differenced time series of log abundance [$\ln(N_t) - \ln(N_{t-1})$, where N_t is the population size at time t] of all patch pairs in the population (Bjornstad et al. 1999). To estimate constancy we calculated the fluctuation index (Dey and Joshi 2006) (FI):

$$FI = \frac{1}{T\overline{N}} \sum_{t=0}^{T-1} Abs(N_{t+1} - N_t)$$

where $\overline{N}$ is the mean population size over T transfers. FI measure the mean one-step change in abundance, scaled by average population size, over the duration of the experiment. FIs were calculated at both the level of the population and the patch (i.e. individual wells). Levels of population synchrony and FI's at both patch and population levels were analysed using two-way ANOVAs, with presence or absence of phages and level of dispersal fitted as factors. Where significant interactions between phage presence and dispersal were detected, simple effects of phage and dispersal were analysed using orthogonal contrasts, using a Bonferroni corrected alpha value of 0.01.

2.4 Results

2.4.1 Bacterial abundance

Population dynamics for representative populations under each experimental treatment are displayed in Figure 2.1. Phages and dispersal had interactive effects on bacterial abundance (Fig. 2.2; phage * dispersal interaction: $F_{2,30} = 3.76$, $P < 0.05$). Phages reduced mean bacterial abundance across all dispersal treatments (Fig. 2.2; simple effect of phages: no dispersal, $F_{1,30} = 36.1$, $P < 0.001$; localized dispersal, $F_{1,30} = 96.0$, $P < 0.001$; global dispersal, $F_{1,30} = 74.4$, $P < 0.001$) and bacterial abundance decreased with increasing dispersal in the presence of phages (Fig. 2.2; simple effect of dispersal: $F_{2,30} = 7.65$, $P < 0.01$) but not in the absence of phages (Fig. 2.2; simple effect of dispersal: $F_{2,30} = 0.09$, $P > 0.01$).

2.4.2 Spatial synchrony

The synchrony of fluctuations in bacterial abundance increased with dispersal for both phage containing (Fig. 2.3; simple effect of dispersal: $F_{2,30} = 68.9$, $P < 0.001$) and phage-free populations (Fig,. 2.3; simple effect of dispersal: $F_{2,30} = 16.2$, $P < 0.001$). However, this effect was far stronger in parasitized compared to

unparasitized populations (Fig. 2.3; phage * dispersal interaction: $F_{2,30} = 11.56$, $P < 0.001$). While phage had no effect on spatial synchrony in populations without dispersal (Fig. 2.3; simple effect of phages: $F_{1,30} = 0.036$, $P > 0.01$), mean population spatial synchrony was significantly higher in parasitized populations with dispersal (Fig. 2.3; simple effect of phages: localized dispersal, $F_{1,30} = 12.7$, $P < 0.01$; global dispersal, $F_{1,30} = 48.8$, $P < 0.001$).

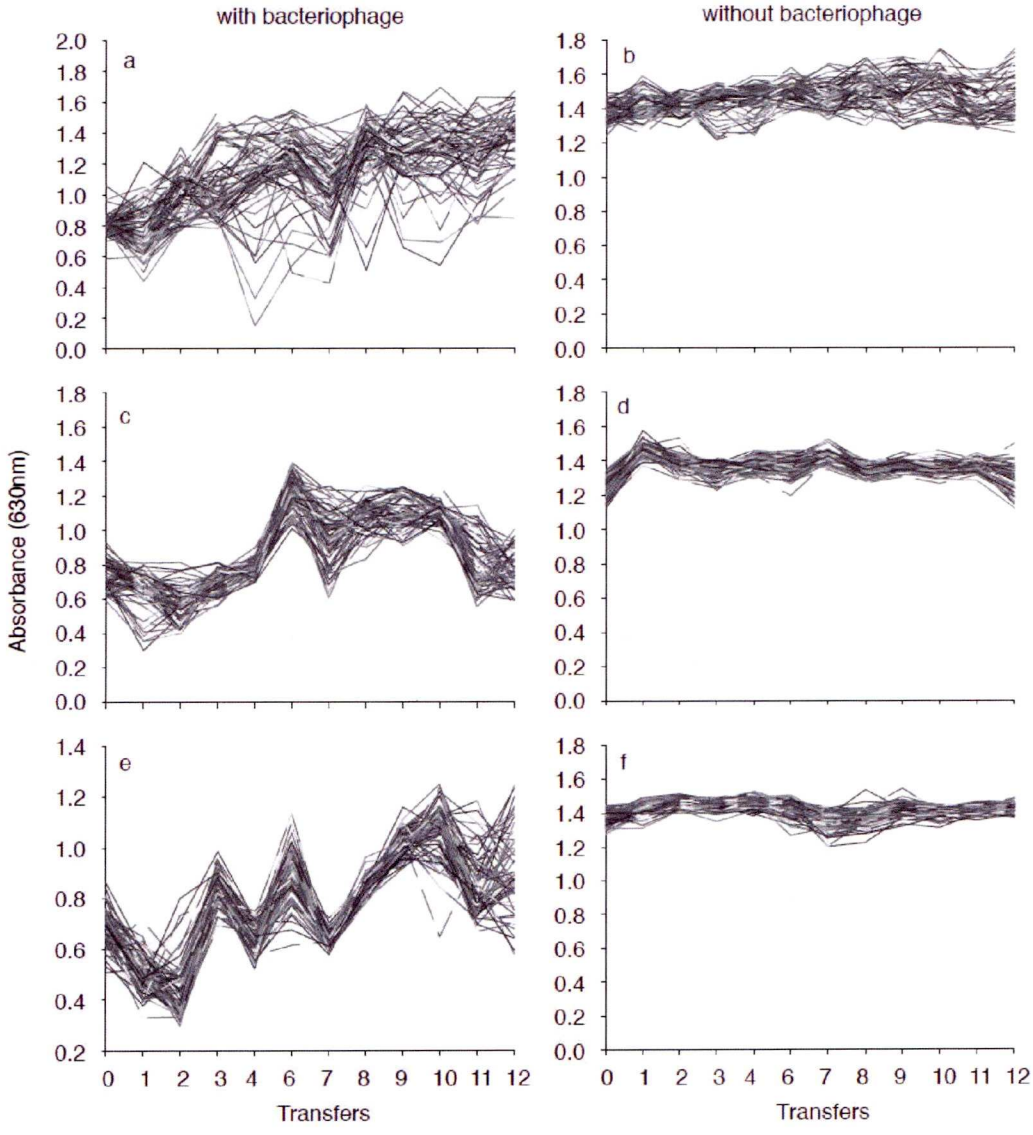


Figure 2.1: Population dynamics

Panels show bacterial abundance dynamics of representative populations under no dispersal (a & b), localized dispersal (c & d) and global dispersal (e & f) in the presence (left-hand panels) and absence (right-hand panels) of bacteriophage parasites. Lines represent bacterial abundance measured as absorbance (630nm) of individual wells.

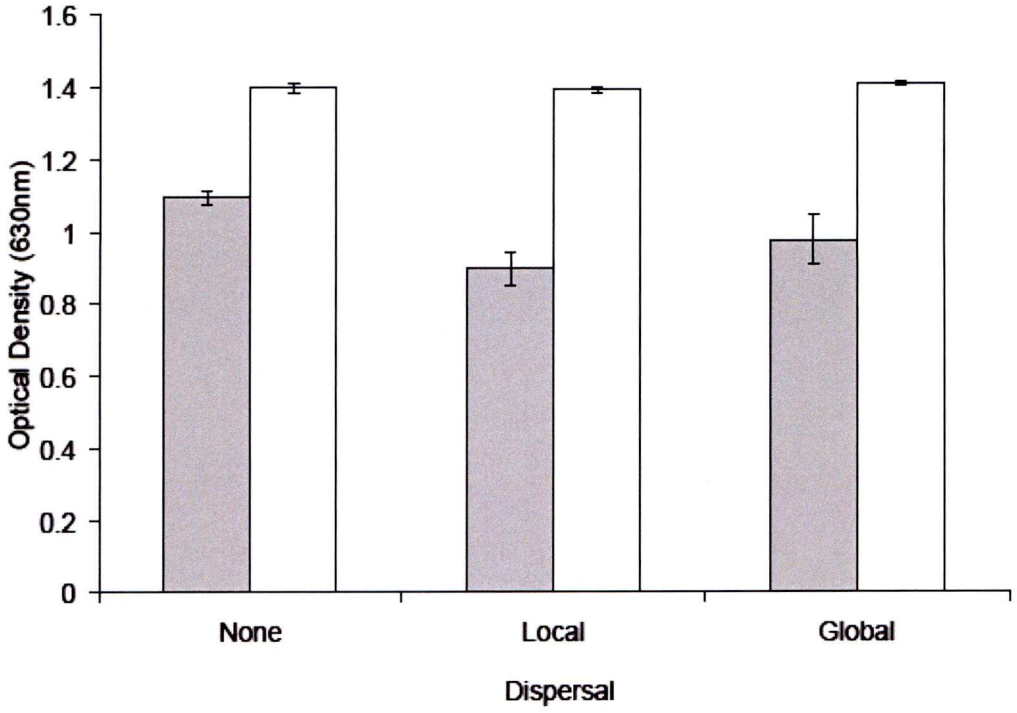


Figure 2.2: Bacterial abundance

Bars represent mean absorbance at 630nm (±SE) in the presence (grey) and absence (white) of bacteriophage parasites.

2.4.3 Patch-level constancy

Analysis of individual patch dynamics revealed that phages and dispersal had interactive effects on patch-level constancy (Fig. 2.4A; phage * dispersal interaction: $F_{2,30} = 12.11$, $P < 0.001$). Patch-level constancy was much lower in the presence of phages, which drove strong within patch fluctuations in bacterial abundance (Fig. 2.4A; simple effect of phages: no dispersal, $F_{1,30} = 113.7$, $P < 0.001$; localized dispersal, $F_{1,30} = 114.3$, $P < 0.001$; global dispersal, $F_{1,30} = 252.6$, $P < 0.001$). In the presence of phages, patch-level constancy decreased with increasing dispersal (Fig. 2.4A; simple effect of dispersal: $F_{2,30} = 5.45$, $P < 0.01$), while, in contrast, patch-level constancy increased with dispersal in the absence of phages (Fig. 2.4A; simple effect of dispersal: $F_{2,30} = 7.14$, $P < 0.01$).

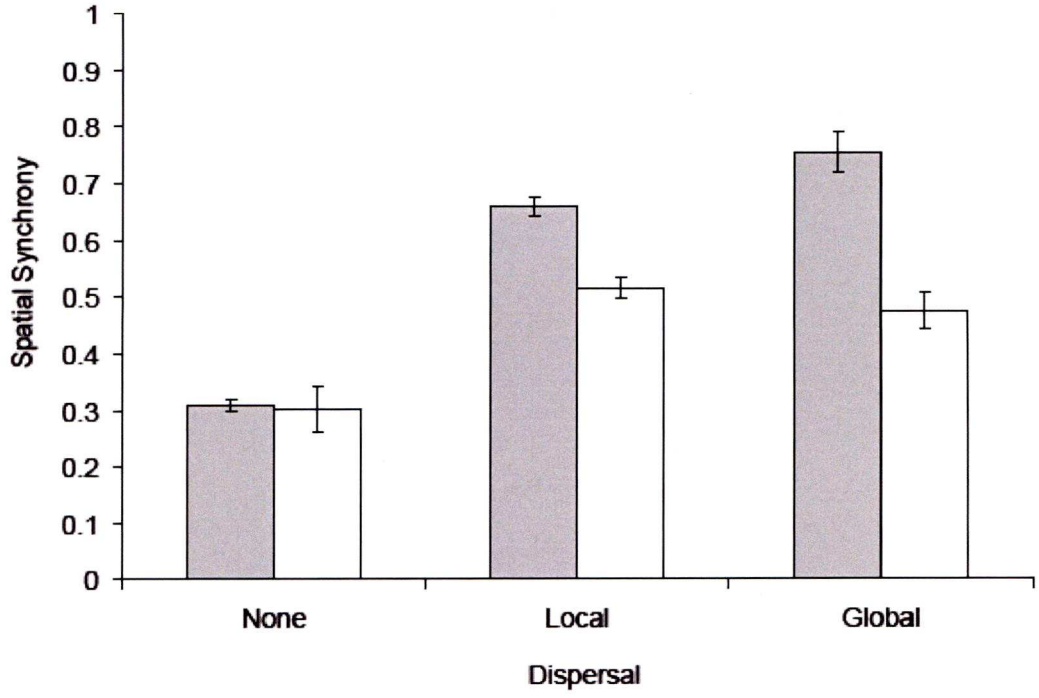


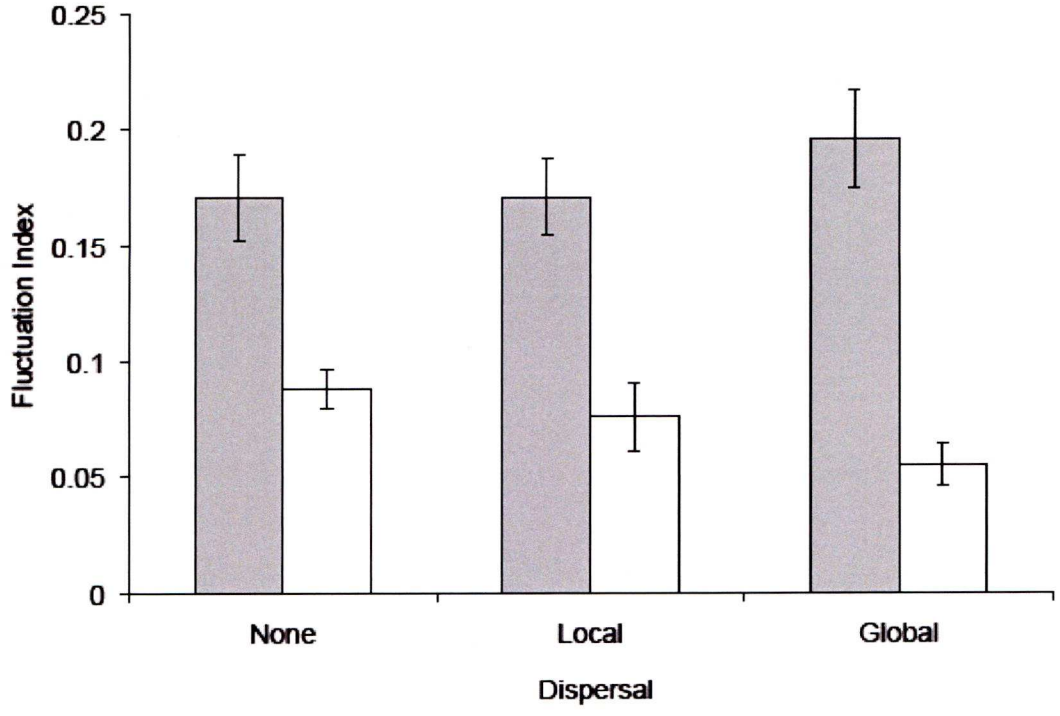
Figure 2.3: Spatial synchrony

Bars represent mean cross correlation coefficients (±SE) in the presence (grey) and absence (white) of bacteriophage parasites

2.4.4 Population-level constancy

Dispersal had contrasting effects on population-level constancy in the presence and absence of phages (Fig. 2.4B; phage * dispersal interaction: $F_{2,30} = 9.79$, $P < 0.001$). Dispersal in the absence of phages had no effect on population-level constancy (Fig. 2.4B; simple effect of dispersal: $F_{2,30} = 0.228$, $P > 0.01$), while dispersal in the presence of phages decreased population-level constancy (Fig. 2.4B; simple effect of dispersal: $F_{2,30} = 15.6$, $P < 0.001$). Overall parasitized populations were much less stable than unparasitized ones (Fig. 2.4B; simple effect of phages: no dispersal, $F_{1,30} = 54.2$, $P < 0.001$; localized dispersal, $F_{1,30} = 60.9$, $P < 0.001$; global dispersal, $F_{1,30} = 92.3$, $P < 0.001$). This was due to phages driving within-patch fluctuations in bacterial abundance that were synchronized by dispersal, becoming phase-locked across the population as a whole thereby reducing population-level constancy.

A



B

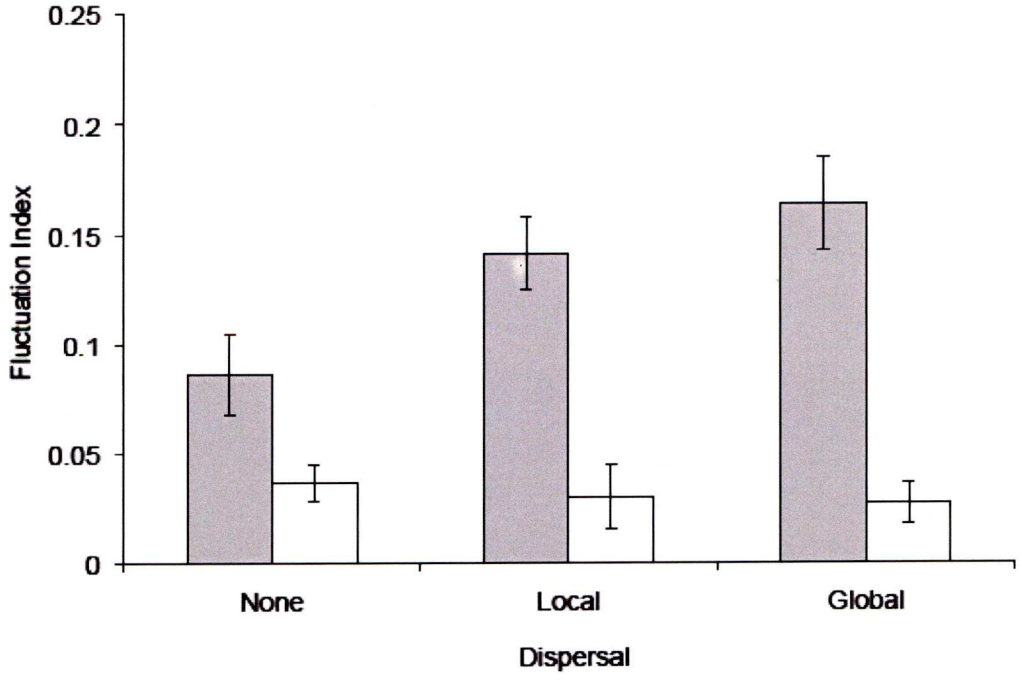


Figure 2.4: Patch-level and population-level constancy

Panels show constancy measured as Fluctuation Index at the patch level (panel A) and population level (panel B). Bars represent mean fluctuation index (±SE) in the presence (grey) and absence (white) of bacteriophage parasites.

2.5 Discussion

Our data suggest that the impact of dispersal on local and regional dynamics depends on the presence of natural enemies. These results are likely due to the ways in which stochastic and deterministic fluctuations interact across the population. In the absence of parasites, population fluctuations would have arisen primarily through demographic stochasticity due to random variation in initial densities and growth rates within each patch. The evolution of novel genotypes by *de novo* mutation over the course of the experiment is also likely to have contributed to demographic variation between patches. Dispersal between these randomly fluctuating patches would only have had a moderate synchronising effect, averaging out stochastic variation. By contrast, the presence of a natural enemy resulted in strong multi-generational, deterministic population cycles that swamped the inherent stochastic fluctuations, such that sub-populations quickly became phase-locked in the presence of dispersal. Hence, each patch across the population showed highly synchronised fluctuations diminishing the potential for rescue effects. Indeed, this is in keeping with theory, which predicts that populations with cyclical dynamics should synchronize more strongly through dispersal than those with non-cyclical dynamics (Bjornstad et al. 1999).

In addition to the ecological dynamics described so far it is likely that fluctuations in bacterial abundance in parasitized populations were also driven by coevolution. Previous studies have shown that *P. fluorescens* and bacteriophage SBW25Φ2 undergo rapid antagonistic coevolution with directional selection for increased resistance and infectivity ranges respectively over the timescale of our experiments (Buckling and Rainey 2002; Brockhurst et al. 2007b). Such coevolution is known to depress bacterial population density through phage-induced mortality following evolution of phage with broader host range (Buckling and Rainey 2002; Buckling and Hodgson 2007). The observed fluctuations in bacterial abundance were likely caused in part by evolutionary changes in resistance and infectivity profiles of bacteria and phage, in addition to classical Lokta-Volterra population dynamics. It is increasingly recognized that rapid evolution can affect ecological dynamics in a wide range of systems (Thompson 1998; Hairston Jr et al. 2005). Indeed, rapid evolution of resistance in bacteria is

likely to have increased persistence by preventing phages from driving bacterial populations extinct.

Hosts and parasites were co-dispersed at equal rates in our experiment. In some host-parasite associations such congruent patterns of host and parasite gene flow are observed (Mulvey et al. 1991). However, in certain others, patterns of host and parasite gene flow are decoupled with either the host (Delmotte et al. 1999) or the parasite (Dybdahl and Lively 1996; Davies et al. 1999) displaying relatively greater levels of gene flow. The potential importance of differential relative rates of dispersal of victims and enemies for ecological dynamics is highlighted by the findings of Huffaker's classic studies of predator and prey mite species (Huffaker 1958). Here the greater persistence stability of populations under increased spatial complexity was thought to be due to the greater dispersal ability of prey relative to predatory mites. Our findings may therefore be limited to host-parasite systems that experience simultaneous host-parasite dispersal. Such situations are likely to arise where the parasite relies upon the host for its dispersal, as is the case for contact transmitted parasites, or where co-dispersal of host and parasite is driven by an external factor such as a prevailing wind or aquatic current.

An additional but important caveat of our experiment is that the rates of dispersal we used were relatively high compared to those commonly observed in natural systems (Slatkin 1985). Our dispersal regimes consisted of mass migration events at each transfer, with each growth period between transfers allowing approximately 10 bacterial generations. This equated to Slatkin's m (i.e., proportion of immigrants per patch per generation (Slatkin 1985)) values of ~ 0.10 for global dispersal and ~ 0.09 for localized dispersal. Our finding that these relatively high rates of dispersal reduced stability of parasitized populations is in line with theory, which predicts a hump-shaped relationship between dispersal rate and population stability (Taylor 1990). This suggests that lower rates of dispersal may have had a stabilizing effect on ecological dynamics in this host-parasite system. By contrast, in the absence of parasites, the rates of dispersal used did not reduce stability of populations. It is possible that natural enemies altered the range of dispersal rates that led to increased population stability, or

alternatively, that dispersal had a weaker impact on ecological dynamics in the absence of natural enemies (Rohani et al. 1996).

These results confirm the importance of biotic factors as drivers of spatial synchrony of ecological dynamics. Dispersal has been shown to increase spatial synchrony in single species (Lecomte et al. 2004) and host-enemy systems (Holyoak and Lawler 1996; Holyoak 2000). However, this is the first experimental evidence that dispersal can have qualitatively different effects on ecological dynamics and population stability of a focal species in the presence and absence of a natural enemy. Specifically, while dispersal increased stability in the absence of the parasite, it decreased stability in the presence of parasites by causing spatially synchronized fluctuations in abundance. It is also of note that in this system, natural enemies and dispersal alone were sufficient to drive spatial synchrony without the need for extrinsic forcing (Cattadori et al. 2005) as has been predicted by theory (Blasius et al. 1999).

Chapter 3: The impact of parasite dispersal on antagonistic host-parasite coevolution

3.1 Abstract

Coevolving populations of hosts and parasites are often subdivided into a set of patches connected by dispersal. Higher relative rates of parasite compared to host dispersal are expected to lead to parasite local adaptation. However, we know of no studies that have considered the implications of higher relative rates of parasite dispersal for other aspects of the coevolutionary process, such as the rate of coevolution and extent of evolutionary escalation of resistance and infectivity traits. We investigated the effect of phage dispersal on coevolution in experimental metapopulations of the bacterium *Pseudomonas fluorescens* SBW25 and its viral parasite, phage SBW25Φ2. Both the rate of coevolution and the breadth of evolved infectivity and resistance ranges peaked at intermediate rates of parasite dispersal. These results suggest that parasite dispersal can enhance the evolutionary potential of parasites through provision of novel genetic variation, but that high rates of parasite dispersal can impede the evolution of parasites by homogenizing genetic variation between patches, thereby constraining coevolution.

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3.2 Introduction

Antagonistic host-parasite coevolution, the reciprocal evolution of enhanced host defence and parasite counter-defence, is pervasive in natural communities and is implicated in a wide range of ecological and evolutionary processes (Woolhouse et al. 2002; Thompson 2005). Often populations of hosts and parasites are subdivided into a set of patches or demes connected by dispersal (a metapopulation). Under such conditions the dynamics and outcomes of coevolution are likely to be influenced by the relative levels of dispersal between patches in each of the interacting species. All else being equal it is predicted that the species with the greater level of dispersal will have the upper hand in a given coevolutionary arms race (Gandon and Michalakis 2002; Greischar and Koskella 2007; Hoeksema and Forde 2008). This arises because dispersal introduces novel genetic variation into the population, thereby enhancing its adaptive potential (Wright 1931). However, theoretical and empirical studies suggest that very high levels of dispersal can have a detrimental effect on genetic variation and thereby adaptive potential (reviewed in Garant et al. 2007). This arises through two mechanisms; first, high rates of dispersal can cause "genetic swamping" by replacing locally adapted alleles with locally maladapted alleles common in the metapopulation as whole (Alleaume-Benharira et al. 2006); second, high rates of dispersal can homogenise genetic variation among patches thus reducing the supply of novel variation attainable through dispersal (Gandon and Michalakis 2002). Combined, these processes lead to the prediction that the rate of adaptation is likely to peak at intermediate rates of dispersal.

A wide range of relative rates of gene flow, which is likely to correlate with dispersal rate, have been observed in natural antagonistic associations (encompassing host-parasite and predator-prey). While some antagonistic associations show remarkably congruent patterns of gene flow (Mulvey et al. 1991; Jerome and Ford 2002), in others, the rate of gene flow experienced by antagonists are decoupled, with either the host / prey (Delmotte et al. 1999; Martinez et al. 1999) or the parasite / predator displaying greater levels of gene flow (Michalakis et al. 1994; Dybdahl and Lively 1996; Davies et al. 1999).

Relatively greater rates of parasite compared to host dispersal or gene flow are likely to underlie patterns of parasite local adaptation (i.e., greater performance on sympatric compared to allopatric hosts) observed in natural populations through provision of genetic variation and thereby enhancement of the adaptive potential of parasites (Lively and Dybdahl 2000; Gandon and Michalakis 2002; Greischar and Koskella 2007). Such local adaptation of organisms causing disease to humans or livestock and crops is of particular concern (Woolhouse et al. 2002), thus an understanding of the coevolutionary impact of greater relative rates of parasite compared to host gene flow is required.

In laboratory studies with bacteria and their viral parasites (phage), where rates of dispersal can be directly manipulated, dispersal between patches has emerged as a key determinant of the outcomes and dynamics of coevolution (Forde et al. 2004; Morgan et al. 2005; Brockhurst et al. 2007b; Forde et al. 2007; Morgan et al. 2007). In the *Pseudomonas fluorescens* SBW25 – SBW25Φ2 association bacteria are locally adapted in the absence of dispersal (i.e., bacteria are more resistant to sympatric compared to allopatric phage populations) (Morgan et al. 2005). Moderate increases in the relative rate of bacterial dispersal (1-10%) have no effect on local adaptation; this is because bacteria already have the upper hand in the coevolutionary arms race. By contrast, moderate increases in the relative rate of phage dispersal (1-10%) reverse patterns of local adaptation such that phages are locally adapted. This arises because dispersal introduces novel genetic variation [genetic variation for both resistance and infectivity has been shown to readily evolve in coevolving populations of *P. fluorescens* and SBW25Φ2 (Poullain et al. 2008)] enhancing the adaptive potential of phage such that, on average, phages have the upper hand in the coevolutionary arms race (Morgan et al. 2005). Mechanisms, such as dispersal, that enhance the adaptive potential of the lagging partner in a coevolutionary association can have a significant impact upon the dynamics of coevolution because such reciprocal evolutionary change may only proceed as rapidly as the slowest adapting partner. Such "warming" of coevolutionary cold-spots (Gomulkiewicz et al. 2000) through increased dispersal of the host (Brockhurst et al. 2007b) or host and parasite simultaneously (Forde et al. 2007; Morgan et al. 2007) has been observed in several studies. However, the effect of greater relative rates of parasite dispersal remains unconsidered.

Although empirical evidence suggests that greater relative rates of parasite compared to host dispersal lead to greater parasite local adaptation (Dybdahl and Lively 1996; Lively and Dybdahl 2000; Morgan et al. 2005), the impact on other aspects of the coevolutionary process such as the rate of coevolutionary change and the extent of coevolutionary escalation remain largely unexplored. The rate of coevolution has been shown to affect genetic diversity and population dynamics in coevolving populations (Buckling & Hodgson 2007; Thompson 2005), while the evolution of more broadly infective parasites has clear implications for disease (Woolhouse et al. 2002). Moreover, when compared to the wide range of relative rates of parasite dispersal observed in natural systems, only a very restricted range of relative rates has thus far been studied using experimental metapopulations (Morgan et al. 2005). To further investigate this we established replicate metapopulations of the common soil bacterium *Pseudomonas fluorescens* SBW25 and its lytic viral bacteriophage SBW25Φ2, which were propagated by serial transfer. Within each metapopulation, phages were migrated from a migrant-pool at a range of different rates representative of those observed in natural systems, ranging from no dispersal in both host and parasite, to increasingly greater dispersal of parasites relative to hosts. Hosts were left unmigrated in all treatments. Previous studies with this host-parasite association have shown persistent cycles of coevolution imposing directional selection for increased infectivity and resistance ranges through time in phage and bacteria respectively (Buckling and Rainey 2002), such ranges are a measure of the extent of coevolutionary escalation. We assayed levels of evolved bacterial resistance range and phage infectivity range (these are "global" measures against both sympatric and allopatric antagonists), as well as the rate of coevolutionary change in one focal patch within each experimental metapopulation.

We hypothesised that phage dispersal would increase the adaptive potential of phages by introducing novel genetic variation, but that high levels of dispersal would impede adaptation by homogenizing genetic variation between patches and / or introducing locally maladaptive alleles. This leads to the prediction of a negative quadratic effect of of phage gene-flow rate on the adaptive potential of phages. Because bacteria are ahead in the coevolutionary arms race in the absence

of dispersal and phage adaptation is the rate-limiting-step of coevolution in this system, we further predicted: [1] a negative quadratic relationship between the rate of coevolution and phage dispersal rate; [2] a negative quadratic relationship of the extent of evolutionary escalation in resistance and infectivity ranges with phage dispersal rate.

3.3 Materials and Methods

3.3.1 Culturing Conditions

Cultures were grown in 30ml glass universals with loose fitting plastic caps containing 6ml of Kings B (KB) medium in a static incubator at 28°C. Cultures were propagated by serial transfer, with 60µl of culture being transferred to a fresh KB microcosm every 48 hours. Samples of each culture were frozen in 20% glycerol and stored at -80°C every two transfers throughout the course of the experiment.

3.3.2 Isolation of bacteria and phage

Phage samples were isolated during the experiment by centrifuging samples of culture (13000 rpm, 2mins) in 10% chloroform. This lysed and pelleted the bacterial cells, leaving a suspension of phage particles in the supernatant. Isolated phage samples were then stored at 5°C. Bacteria were isolated by growing cultures overnight in a KB microcosm containing 0.37% Virkon® (a commercially available disinfectant). At this concentration Virkon® is toxic towards bacteriophage particles while being non-toxic towards *P. fluorescens*. 60µl was then transferred to a fresh KB microcosm and incubated for a further 24 hours. This treatment left bacteria viable and free from phage and Virkon®. Presence of phage following this procedure was routinely checked by assaying the infectivity of a sample of culture against ancestral bacteria, no phages were detected.

3.3.3 Initiating Populations

18 KB microcosms were inoculated with approximately 10^7 isogenic cells of *P. fluorescens* isolate SBW25 and 10^5 isogenic particles of the lytic DNA phage, SBW25Φ2. Cultures were initially propagated for 8 transfers to allow divergence between populations prior to migration.

3.3.4 Experimental treatments

Following divergence, populations were assigned to one of six replicate metapopulations, each consisting of three microcosms. Each replicate metapopulation was then used to found five further metapopulations, each of which was subjected to one of five different phage migration regimes (0%, 0.1%, 1%, 10% and 100% of phage population from migrant-pool) for 24 days (12 transfers) of culturing. Bacteria were left unmigrated in all treatments: at each transfer samples of bacteria were isolated from each population and 60µl of this isolate was transferred to a fresh microcosm. By contrast transferred phage came from two sources: unmigrated phage isolated from the relevant population, and phage from a migrant-pool for each metapopulation, which consisted of equal proportions by volume of phages isolated from each constituent microcosm. The proportion of the total transferred volume (60µl) added from each source was determined by the migration treatment, for example under the 1% migration regime, 0.6µl of transferred phage came from the migrant pool and 59.4µl came from the phage isolated from the relevant population.

3.3.5 Assays

3.3.5.1 Quantifying resistance and infectivity

Bacterial resistance was assayed as a binary trait, such that a given bacterial colony could be either susceptible or non-susceptible to infection by phage. For each assayed population, ten individual bacterial colonies were isolated by plating on a KB agar plate. Colonies were then streaked across a 20µl line of phage on a

KB agar plate and incubated for 24 hours at 28°C. A colony was defined as susceptible if there was visible inhibition of growth upon crossing the line of phage. Resistance was recorded as the proportion of non-susceptible bacteria per population, while infectivity was measured as the proportion of susceptible bacteria per population. Within each migration treatment, one focal population from each of the six replicate metapopulations was selected to undergo assays.

3.3.5.2. Rate of coevolution

To determine if directional antagonistic coevolution occurred in this experiment, we used stored population samples (see above) to measure how the infectivity of phage populations to a bacterial population changed through time. Specifically, at transfers 2, 4, 6, 8 and 10 we determined the resistance (proportion resistant colonies) of bacterial populations to past (two transfers previous), contemporary and future (two transfers subsequent) sympatric phage populations. If directional antagonistic coevolution was occurring then we would expect, for multiple time points, future phage to be better than contemporary phage, and for contemporary phage to be better than past phage at infecting contemporary bacteria, hence a positive slope of infectivity against time (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, and averaged across time-points (Brockhurst et al. 2003). Because bacterial resistance to contemporary phage remains relatively constant across time-points, we can infer bacterial adaptation (Brockhurst et al. 2003; Brockhurst et al. 2007b), hence when considered over multiple time-points this is a measure of coevolution, rather than simply phage infectivity evolution.

3.3.5.3. Resistance and infectivity ranges

The breadth of resistance and infectivity ranges was assayed every four transfers by determining the resistance / infectivity for each bacteria / phage population when assayed against all other focal populations from the other migration treatments that shared a founding metapopulation. This provides a "global"

measure of which treatment has produced the relatively most infectious and resistant populations, while controlling for the effect of founding metapopulation. Phage infectivity to their sympatric bacteria (i.e. the bacteria from the same microcosm and time point) was measured every two transfers throughout the course of the experiment.

3.3.6 Statistical analysis

Sympatric infectivity, rate of coevolution and breadth of infectivity and resistance ranges were averaged through time and analysed separately using General Linear Models (GLM) carried out in Minitab. Founding metapopulation was fitted as a random factor and Log10 (migration rate + 0.01) was simultaneously fitted as both a linear and quadratic covariate. Whether the addition of a quadratic term significantly improved model fit over a simpler linear model was determined using partial F-tests. Resistance ranges through time were log10 transformed and infectivity ranges through time were square-root transformed to meet the necessary assumptions (normality, homogeneity of variance).

3.4 Results

As predicted, we observed a negative quadratic relationship between the rate of phage dispersal and the rate of coevolution which peaked at 1% (Figure 3.1; founding metapopulation, $F_{5,22} = 0.77$, $P = 0.579$; linear effect, $F_{1,22} = 0.37$, $P = 0.550$; negative quadratic effect, $F_{1,22} = 7.29$, $P = 0.013$, partial F-test for inclusion of quadratic rate term, $F_{1,22} = 7.29$, $P < 0.05$). Because coevolution is predominantly directional in this system (Buckling and Rainey 2002), more rapid coevolution is typically associated with the evolution of broader phage infectivity range. In line with this, a negative quadratic relationship between the rate of phage dispersal and phage infectivity range was observed which also peaked at 1% (Figure 3.2; founding metapopulation, $F_{5,22} = 3.26$, $P = 0.024$; linear term, $F_{1,22} = 26.18$, $P < 0.001$; negative quadratic term, $F_{1,22} = 38.51$, $P < 0.001$; partial F-test for inclusion of quadratic rate term, $F_{1,22} = 38.5$, $P < 0.01$). These data are consistent with the hypothesis that phage dispersal between patches can increase

genetic variation thereby enhancing phage evolutionary potential, but that high levels of dispersal (10-100%) may impede phage evolution, either through "genetic swamping" or homogenisation of genetic variation between patches, thereby limiting the rate and extent of coevolution attainable.

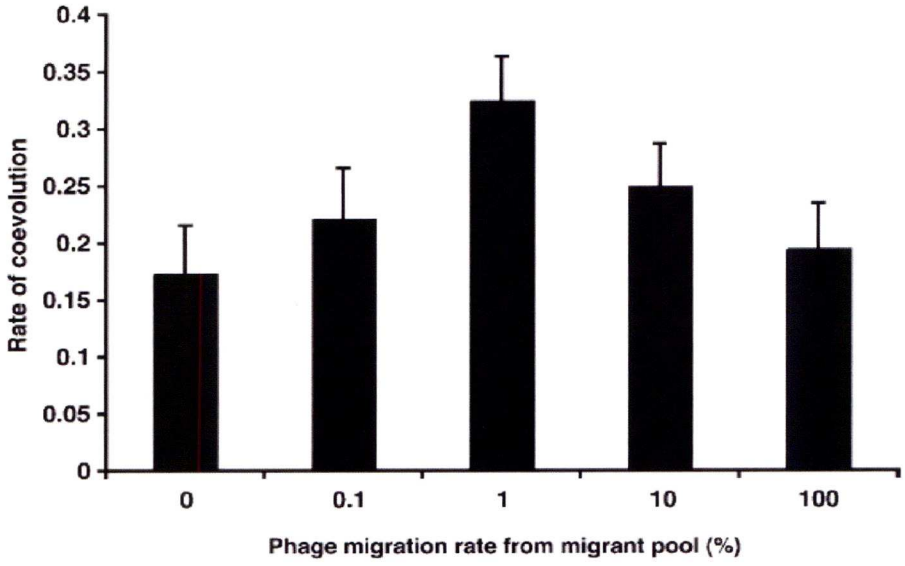

Figure 3.1: The effect of phage migration rate on the rate of coevolution. The rate of coevolution was given by the slope of the change in infectivity of a phage population through time. Bars show mean (+ SEM) rate of coevolution averaged through time.

Because coevolution is a reciprocal process, bacterial resistance range was expected to evolve in response to changes in phage infectivity range. Bacterial resistance ranges also displayed a negative quadratic relationship with the rate of phage migration peaking at 1% (Figure 3.3; founding metapopulation, $F_{5,22} = 2.96$, $P = 0.034$; linear term, $F_{1,22} = 8.94$, $P = 0.007$; negative quadratic term, $F_{1,22} = 9.91$, $P = 0.005$; partial F-test for inclusion of quadratic rate term, $F_{1,22} = 9.94$, $P < 0.01$) and were positively correlated with infectivity ranges (correlation of infectivity and resistance range means; Pearson's $r = 0.935$, $P = 0.02$). This suggests that bacterial resistance ranges were able to successfully evolve in response to the broadening of phage infectivity range through time despite a

complete lack of dispersal. Taken together with previous studies (Morgan et al. 2005; Brockhurst et al. 2007a; Morgan et al. 2007) this suggests that bacterial populations possess potential for coevolutionary escalation that remains unutilized in coevolving populations limited by the rate of phage adaptation.

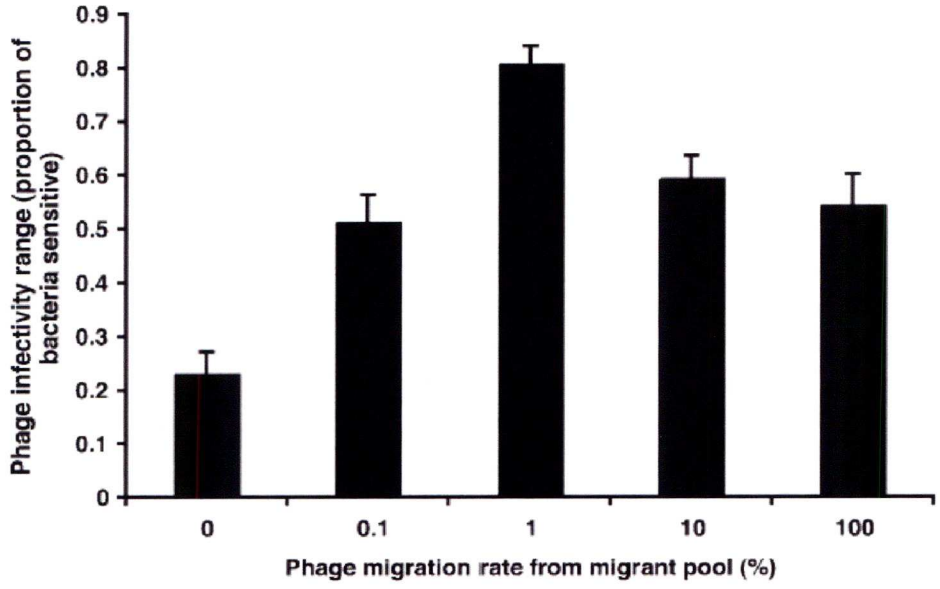

Figure 3.2: The effect of phage migration rate on phage infectivity range. The infectivity range was given by determining the infectivity of each phage population when assayed against bacteria from all other focal populations from the other migration treatments that shared a founding metapopulation, providing a measure of "global" infectivity. Bars show mean (+ SEM) infectivity range of phage populations averaged through time.

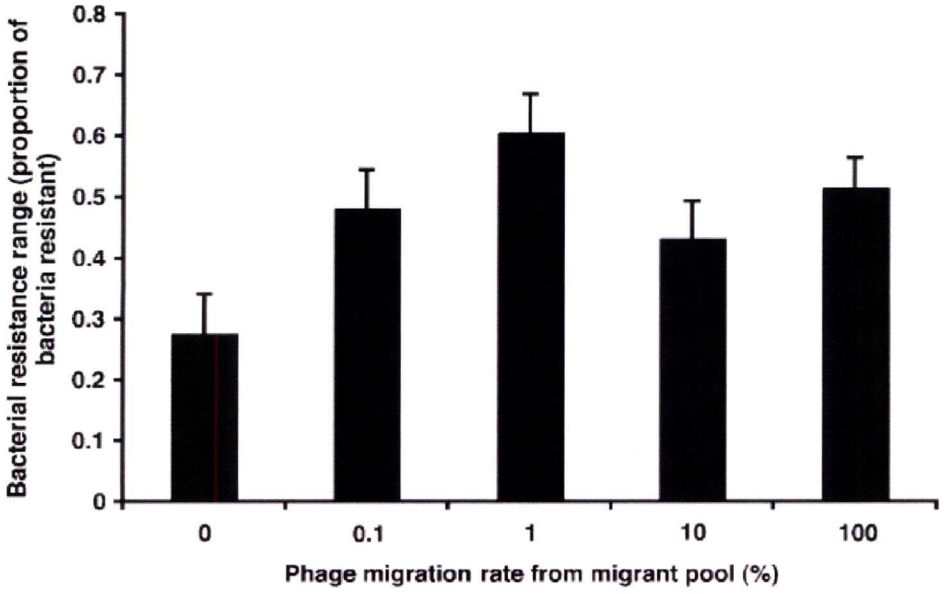

Figure 3.3: The effect of phage migration rate on bacterial resistance range. The resistance range was given by determining the resistance of each bacteria population when assayed against phage from all other focal populations from the other migration treatments that shared a founding metapopulation, providing a measure of "global" infectivity. Bars show mean (+ SEM) resistance range of bacterial populations averaged through time.

The decline in the rate of coevolution and breadth of phage infectivity range at high rates of dispersal (10-100%) could have arisen through two mechanisms: "genetic swamping" causing loss of locally beneficial alleles, or homogenization of genetic variation between patches. To assess whether locally beneficial infectivity alleles were lost at high phage gene-flow rates, infectivity of phages to their sympatric bacterial hosts was analysed; a decline in sympatric infectivity at high rates of dispersal would have been expected if locally beneficial infectivity alleles were being lost through "genetic swamping". However, phage dispersal led to higher levels of infectivity of phages on their contemporary sympatric bacterial hosts (Figure 3.4; founding metapopulation, $F_{5,22} = 3.31$, $P = 0.022$; linear effect, $F_{1,22} = 4.00$, $P = 0.058$; negative quadratic effect, $F_{1,22} = 7.97$, $P = 0.010$; partial F-test for inclusion of quadratic rate term, $F_{1,22} = 7.97$, $P < 0.01$). Further analysis, excluding the 0% dispersal data, found no difference in sympatric infectivity between other rates of dispersal (Figure 3.4; founding metapopulation, $F_{1,16} =$

3.29, P = 0.031; linear effect, $F_{1,16} = 0.30$, P = 0.593; negative quadratic effect, $F_{1,16} = 0.00$, P = 0.955). Because no decline in sympatric infectivity was observed with increasing dispersal rate, this suggests that, in this experimental system, high rates of dispersal do not significantly limit phage adaptation to local bacterial hosts through "genetic-swamping". It seems likely therefore that the decline in the rate of coevolution and breadth of infectivity range observed at high rates of phage dispersal were due to homogenisation of genetic variation between patches.

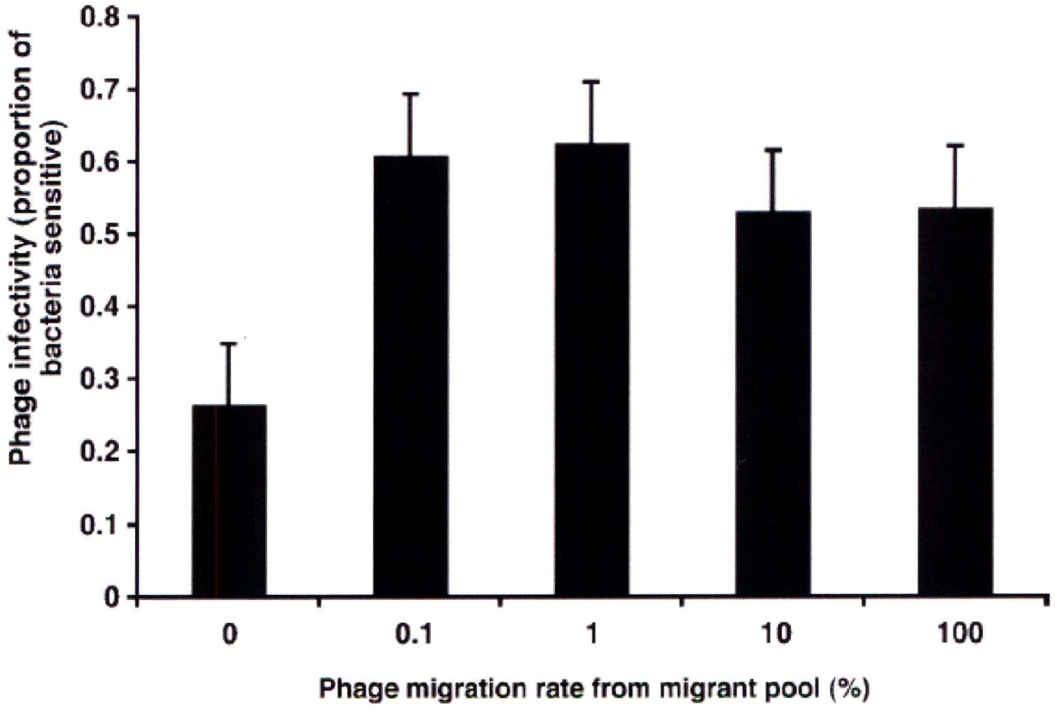

Figure 3.4: The effect of phage migration rate on sympatric infectivity. The sympatric infectivity was given by determining the infectivity of a phage population on bacteria from the same time-point and microcosm. Bars show mean (+ SEM) infectivity of phage populations to contemporary sympatric bacterial populations averaged through time.

3.5 Discussion

Evidence from theory (Gandon and Michalakis 2002), natural populations (Dybdahl and Lively 1996; Lively and Dybdahl 2000) and laboratory populations (Morgan et al. 2005) suggests that greater relative rates of dispersal in parasites

compared to hosts should increase parasite local adaptation (Greischar and Koskella 2007; Hoeksema and Forde 2008). However, local adaptation provides only a contemporary "snap-shot" of coevolutionary interactions, yielding little information about other aspects of the coevolutionary process. The results presented here extend local adaptation findings to consider the effect of a wide range of rates of parasite dispersal on the dynamics and outcomes of coevolution. We demonstrate that parasite dispersal can enhance the evolutionary potential of parasites increasing both the rate and extent of escalation attainable during antagonistic host parasite coevolution. However, high rates of parasite dispersal can impede parasite evolution, our results suggest that the most likely mechanism for this is through homogenizing genetic variation between patches, thereby constraining the coevolutionary process. In a previous study where bacteria and phage were migrated simultaneously (Morgan et al. 2007), evolved phage infectivity range did not decline at high rates of dispersal (10-50%) as observed here. This suggests that the decoupling of host and parasite dispersal can alter the outcome of coevolution by limiting the effects of dispersal on evolutionary potential to one or other antagonist.

Our results suggest that bacterial populations possess coevolutionary potential that remains unutilized in the absence of phage dispersal, posing the question: why if broader resistance ranges can be evolved do they not evolve in the absence of phage dispersal (as seen by the low evolved resistance ranges for 0% migration rate in Fig 3.3)? The strong positive correlation between resistance range and infectivity range in this experiment suggests that selection favours the evolution of sufficient rather than maximal resistance ranges. This is likely to be due to the high cost of phage resistance mutations in this system (Brockhurst et al. 2004; Buckling et al. 2006), such that at any given time bacterial clones with broader than necessary resistance mutations are likely to be outcompeted by sufficiently resistant but fitter clones.

Acceleration of coevolution due to parasite dispersal is likely to be particularly apparent in coevolutionary systems where parasites are the lagging antagonist in the absence of dispersal. This is due to the rate of coevolutionary change being limited by the adaptive rate of the slowest partner. Under such conditions

dispersal is likely to lead to the more rapid evolution of more infective parasites. The generality of the patterns of infectivity and resistance range evolution observed in this study may be somewhat limited to systems that undergo predominantly directional selection. Such systems include most plant-pathogen interactions and other host-parasite interactions that broadly comply with a multilocus gene-for-gene model of coevolutionary interaction, which allows for the evolution of generalist resistance and infectivity phenotypes in hosts and parasites respectively (Thompson and Burdon 1992; Damgaard 1999; Sasaki 2000).

In this and previous studies with this host – parasite association, adaptation has consistently peaked at 1% dispersal despite differences in the precise ecological conditions used in each study (Brockhurst et al. 2007a; Morgan et al. 2007). However, it is unclear how low, intermediate or high rates of dispersal should be defined for natural systems. Undoubtedly this is likely to be under the influence of a wide range of contributory factors that also affect genetic diversity (e.g., mutation rate, population size generation time, etc.). Given this proviso, these results could have implications for health and agriculture. Moderate increases in parasite dispersal associated with increased mobility of human populations and movement of livestock and crops could significantly alter coevolutionary dynamics leading to the more rapid emergence of more infective parasites. Both theoretical and empirical evidence suggests that through increasing transmission opportunities this is likely to be associated with an increase in the virulence of disease (Herre 1993; Boots and Sasaki 1999; Boots et al. 2004; Boots and Mealor 2007). By contrast, very large increases in parasite dispersal rate are likely to erode the potential benefits to parasites of dispersal leading to decline of parasite evolutionary potential, thereby limiting infectivity and virulence evolution.

Chapter 4: How does spatial dispersal network affect the evolution of parasite local adaptation?

4.1 Abstract

Studying patterns of parasite local adaptation can provide insights into the spatio-temporal dynamics of host-parasite coevolution. Many factors both biotic and abiotic have been identified that influence parasite local adaptation. In particular, dispersal and population structuring are considered important determinants of local adaptation. We investigated how the shape of the spatial dispersal network within experimental landscapes affected local adaptation of a bacteriophage parasite to its bacterial host. Regardless of landscape topology, dispersal always led to the evolution of phages with broader infectivity range. However, when the spatial dispersal network resulted in spatial variation in the breadth of phage infectivity range, significant levels of parasite local adaptation and local maladaptation were detected within the same landscape. This empirically confirms theoretical expectations that geographic mosaics may play an important role in driving parasite local adaptation, particularly when the shape of the dispersal network generates non-uniform levels of host resistance or parasite infectivity throughout a species' range.

4.2 Introduction

Studying patterns of parasite local adaptation can reveal the spatio-temporal dynamics of host-parasite coevolution (Thrall et al. 2002). Historically, parasites were often predicted to have an evolutionary advantage over their hosts, due to their generally shorter generation times and larger population sizes, and as such were expected to be locally adapted (i.e., able to overcome immune responses of local hosts) (Price 1980; Ebert 1994). However, empirical studies have revealed that this is not always the case and examples of parasite local maladaptation or no spatial variation in adaptation are common in the literature (reviewed in Greischar and Koskella 2007; Hoeksema and Forde 2008).

Fundamentally, local adaptation in host-parasite systems is driven by spatially asynchronous coevolutionary dynamics, and is therefore influenced by the degree of dispersal between populations (Frank 1991; Gandon et al. 1998). In the absence of dispersal, this asynchrony can be maintained by the coevolutionary process itself producing spatially variable selection (Gandon et al. 1998; Gandon 2002). In the presence of dispersal, asynchronous allele frequencies are still predicted to be maintained under certain conditions, despite dispersal acting as a synchronising force (Gandon and Nuismer 2009). This can be via either stochasticity, with small population sizes coupled with genetic drift preventing synchrony (Burdon 1992; Thompson and Burdon 1992; Gandon 2002), or by deterministic processes but under somewhat stricter conditions – low migration, many populations and strong selection (Gavrilets and Michalakis 2008; Gandon and Nuismer 2009). Alternatively, selection mosaics, where the strength of the reciprocal selection between host and parasite is spatially variable (Thompson 2005), are predicted to be able to prevent homogenisation by selecting for differing alleles in different parts of a species' range (Nuismer 2006; Gandon and Nuismer 2009).

Although dispersal is known to play a central role in the evolution of local adaptation (Greischar and Koskella 2007), the role that landscape topology, or the spatial dispersal network, plays in driving the evolution of local adaptation during host-parasite coevolution has been largely overlooked. The arrangement of

subpopulations or patches within a landscape affects its connectivity (Bull et al. 2006) – the number or the identity of the populations that are connected by dispersal. As such, it can influence the diversity of migrants a patch receives, which is considered a major determinant of the evolutionary potential of a population (Gandon and Michalakis 2002; Garant et al. 2007; Morgan et al. 2007; Vogwill et al. 2008). Furthermore, certain arrangements of patches may result in some patches receiving greater diversity of migrants than other patches within the same landscape, potentially affecting evolutionary potential between patches within landscapes. Here we manipulate the topology of 6-patch experimental landscapes consisting of the common soil bacterium *Pseudomonas fluorescens* SBW25 and its lytic bacteriophage parasite SBW25Φ2 (Buckling and Rainey 2002; Brockhurst et al. 2007b), to investigate how different landscape topologies affect host-parasite coevolution and, in particular, levels of parasite local adaptation.

4.3 Materials and Methods

4.3.1 Initiating Populations

24 microcosms were inoculated with approximately 10^7 isogenic cells of *P. fluorescens* isolate SBW25 and 10^5 isogenic clonal particles the lytic DNA phage, SBW25Φ2. Microcosms consisted of a 30ml glass universal with loose fitting plastic caps containing 6ml of Kings B (KB) medium grown in a static incubator at 28°C. Cultures were propagated by serial transfer, whereby 60µl of culture (1% of the population) was transferred to a fresh KB microcosm every 48 hours. Cultures were initially propagated for 8 experimental transfers to allow divergence between populations. Cultures were then grouped into four experimental landscapes of six microcosms each. Each of these initial landscapes was used to found four replicate landscapes, each of which was exposed to a different landscape shape and propagated for a further twelve experimental transfers.

4.3.2 Experimental Design

Experimental transfers after the initial divergence also involved the transfer of 60µl of culture to a fresh microcosm, but migration was simulated by 1% of this inoculum coming from other populations within the same experimental landscape. Which population provided these migrants depended on the topology of the landscape. Four different landscape topologies were used: isolated, linear, circular and global. In the Isolated treatment populations were maintained without any migration between populations within a landscape. Migration in the linear dispersal treatment consisted of uni-directional stepping-stone migration along a linear string of populations, whereby populations received migrants from the population immediately upstream of them at each transfer. Similarly, the circular dispersal treatment consisted of uni-directional stepping-stone migration along a linear string, but the string was wrapped so that the end of the string connected to the beginning. In the global dispersal treatment all populations contributed to a pool of migrants that were redistributed to all patches at every transfer.

4.3.3 Sampling Populations

Cultures were frozen every two transfers throughout the course of the experiment in 20% glycerol and stored at -80°C. Phage populations were isolated by centrifuging in 10% chloroform and then stored at 5°C. Bacterial populations were isolated by plating on agar.

4.3.4 Infectivity Assays

Infectivity of a particular phage population against a particular bacterial population is here defined as the proportion of bacterial colonies that the bacteriophages are capable of infecting (the proportion of susceptible bacteria). Similarly, bacterial resistance is measured as the proportion of colonies which are resistant. This is assayed by first drying a line of the phage population onto an agar plate, and then streaking 10 bacterial colonies perpendicularly across it.

Bacteria are deemed susceptible if they suffer any reduction in growth upon encountering the line.

Streaking assays were used to measure levels of phage infectivity within landscapes. Populations of phage and bacteria were crossed against each other from positions 2, 4 and 6 within each metapopulation after 2, 4, 6, 8, 10 and 12 experimental transfers. Populations 2,4 and 6 represent the 2^{nd}, 4^{th} and 6^{th} population along the string for linear landscapes, and were selected on this basis so that population 1, which receives no gene flow, would not be assayed. Comparisons between populations receiving no dispersal and some dispersal are common in the literature, and this was not the goal of this manuscript. Numbering of populations is arbitrary in other landscapes, but is based on having a shared founding population with the corresponding population from the linear landscape.

Overall phage infectivity range is measured as the average level of infectivity against all host assay populations from within its own landscape. Likewise, the average resistance range of a bacterial population to all phage assay populations within its own landscape it used as a measure of overall bacterial resistance. Levels of parasite local adaptation were also calculated from the streaking assays. Both commonly used definitions of local adaptation (Kaltz and Shykoff 1998; Kawecki and Ebert 2004; Hoeksema and Forde 2008) were used: home against away and local against foreign. In the former this is comparing 'local' phage performance on 'home' hosts against 'local' phage performance on 'away' hosts, while in the latter it is comparing 'local' phage performance on 'home' hosts against 'foreign' phage performance on 'home' hosts.

4.3.5 Statistical Analysis

To detect within-metapopulation variation in resistance and infectivity ranges, levels of overall host resistance range and phage infectivity range were averaged across all six time points and analysed using a general linear model, with position within landscape fitted as a fixed factor and experimental landscape identity as a random factor. The differing definitions of local adaptation were analysed

independently using repeated measures linear mixed models performed in SPSS. For the local versus foreign definition of local adaptation, host population was used as the subject and phage infectivity was used as the repeated measure; phage origin and position-within-landscape were treated as fixed factors and experimental landscape identity as a random factor. For the home versus away definition, phage population was used as the subject and phage infectivity was used as the repeated measure; host origin and position-within-landscape were treated as fixed factors and experimental landscape identity as a random factor. Degrees of freedom were estimated using the Satterthwaite approximation.

4.4 Results

4.4.1 Patterns of Infectivity and Resistance Range

With no dispersal, phage infectivity ranges were relatively low compared to bacterial resistance ranges (figure 4.1A), but with no consistent variation by position within in landscape for either infectivity ranges (table 4.1; position: $F_{2,6} = 0.02$, $P = 0.978$) or resistance ranges (table 1; position: $F_{2,6} = 0.91$, $P = 0.451$). Conversely, infectivity ranges were generally higher than bacterial resistance ranges for populations exposed to global dispersal or circular dispersal (figures 4.1B and 4.1C), but again there was no variation with position in either infectivity range (table 1; position in global treatment: $F_{2,6} = 0.32$, $P = 0.738$; Position in circular treatment: $F_{2,6} = 0.31$, $P = 0.743$) or resistance range (table 4.1; position in global treatment: $F_{2,6} = 0.03$, $P = 0.968$; Position in circular treatment: $F_{2,6} = 0.43$, $P = 0.670$). However, infectivity ranges were found to significantly increase with linear dispersal in the same direction as dispersal (figure 4.1D, table 4.1; Position: $F_{2,6} = 15.30$, $P < 0.01$). In contrast, no increase in bacterial resistance ranges was observed with increasing distance from the beginning of the landscape (figure 4.1D, table 1; Position: $F_{2,6} = 1.12$, $P = 0.386$).

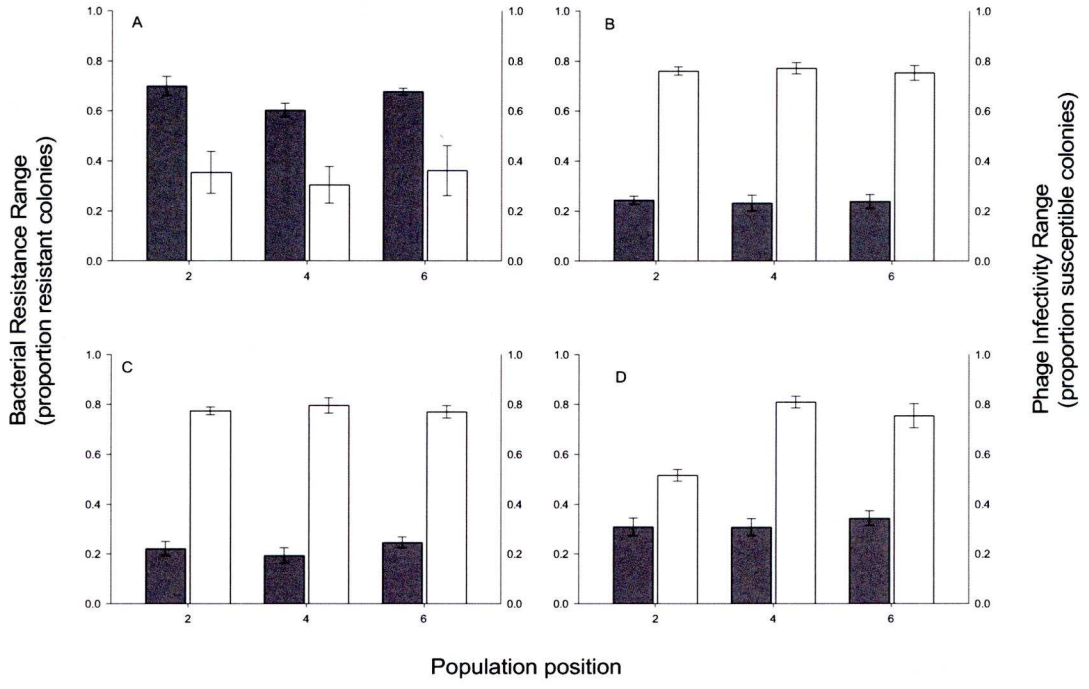

Figure 4.1: Infectivity and resistance: Levels of bacterial resistance (grey bars, proportion resistant colonies ± standard error) and phage infectivity (white bars, proportion susceptible colonies ± standard error) from positions 2, 4 and 6 within landscapes. Panel (A) no dispersal; (B) global dispersal; (C) circular dispersal; (D) linear dispersal.

Table 4.1: Variation in host resistance and phage infectivity by position within landscape. Results of general linear model with position within landscape as a fixed factor, and experimental landscape identity as a random factor.

Resistance				
Dispersal Network		**D.F.**	**F**	**P**
None	**Position**	2,6	0.91	0.451
	Replicate	3,6	1.36	0.342
Global	**Position**	2,6	0.03	0.968
	Replicate	3,6	5.95	**< 0.05**
Circular	**Position**	2,6	0.43	0.67
	Replicate	3,6	3.81	0.077
Linear	**Position**	2,6	1.12	0.386
	Replicate	3,6	9.41	**< 0.05**
Infectivity				
Dispersal Network		**D.F.**	**F**	**P**
None	**Position**	2,6	0.02	0.978
	Replicate	3,6	0.09	0.963
Global	**Position**	2,6	0.32	0.738
	Replicate	3,6	21.34	**< 0.005**
Circular	**Position**	2,6	0.31	0.743
	Replicate	3,6	9.64	**< 0.05**
Linear	**Position**	2,6	15.3	**< 0.01**
	Replicate	3,6	6.73	**< 0.05**

4.4.2 Local versus Foreign Local Adaptation

No consistent variation in the ability of local and foreign phages to infect local hosts was detected for no-dispersal landscapes (phage origin: $F_{1,17} = 0.28$, $P = 0.607$; position: $F_{2,17} = 0.26$, $P = 0.776$; interaction: $F_{2,17} = 0.24$, $P = 0.792$; figure 4.2A). Similarly, no variation in the performance of local and foreign phages on local hosts was detected for either circular or global dispersal (figures 4.2B and 4.2C), with both landscapes producing generally highly infectious phages (global dispersal: phage origin: $F_{1,17} = 1.20$, $P = 0.291$; position: $F_{2,15} = 0.06$, $P = 0.940$; interaction: $F_{2,15} = 0.27$, $P = 0.766$. Circular dispersal: phage origin: $F_{1,15} = 0.65$, $P = 0.434$; position: $F_{2,15} = 0.84$, $P = 0.451$; interaction: $F_{2,15} = 0.07$, $P = 0.936$). However significant differences were detected between local and foreign phage infectivity against local hosts from landscapes subjected to linear dispersal (figure

4.2D), but this depended on the population's position within the string (phage origin: $F_{1,13} = 2.50$, $P = 0.136$; position: $F_{2,13} = 0.76$, $P = 0.489$; interaction: $F_{2,13} = 7.73$, $P < 0.01$). Specifically, phage populations in position 2 tended to be locally maladapted, while populations 4 and 6 both contained locally adapted phage populations.

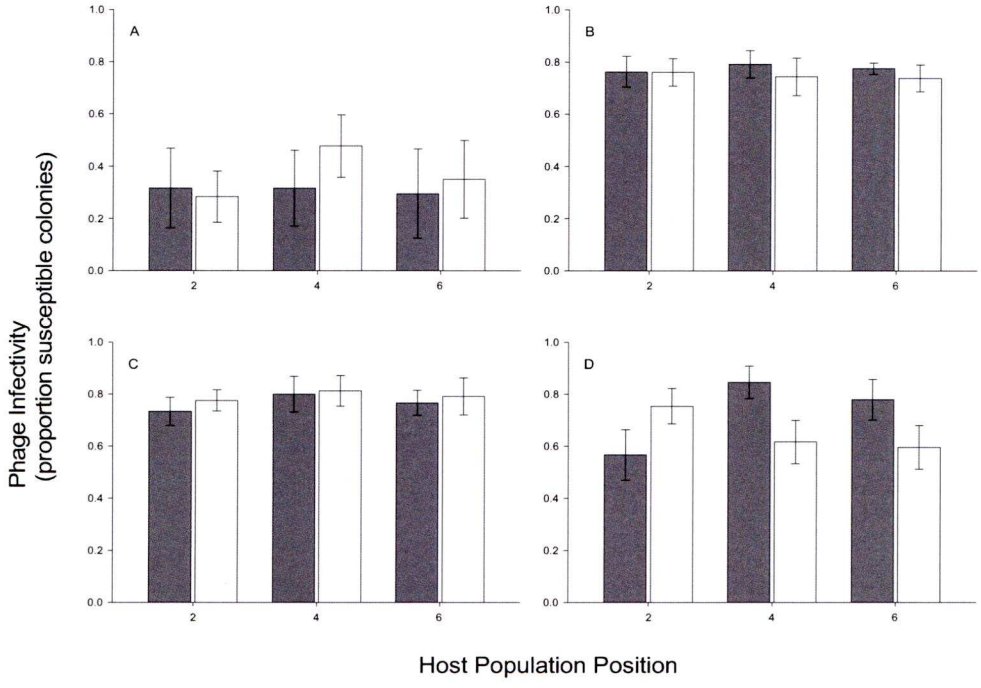

Figure 4.2: Local versus foreign infectivity. Infectivity (proportion susceptible colonies ± standard error) of local phages (grey bars) and foreign phages (white bars) against host population from positions 2,4 and 6 within landscapes. Panel (A) no dispersal; (B) global dispersal; (C) circular dispersal; (D) linear dispersal.

4.4.3 Home versus Away Local Adaptation

No significant variation in the ability of phages to infect home or away hosts from no-dispersal landscapes was detected (figure 4.3A; host population: $F_{1,17} = 0.17$, $P = 0.685$; position: $F_{2,17} = 0.06$, $P = 0.941$; interaction: $F_{2,17} = 0.10$, $P = 0.907$). Nor was there any variation in infectivity against home or away hosts for phage

populations from either global or circular dispersal treatments (figures 4.3B and 4.3C; global dispersal: host population: $F_{1,13} = 1.57$, $P = 0.234$; position: $F_{2,13} = 0.22$, $P = 0.806$; interaction: $F_{2,13} = 0.07$, $P = 0.936$. circular dispersal: host population: $F_{1,14} = 1.04$, $P = 0.326$; position: $F_{2,14} = 0.39$, $P = 0.685$; interaction: $F_{2,14} = 0.87$, $P = 0.440$), with high levels of infectivity against both. Neither was there significant home versus away local adaptation for phages with linear dispersal (Figure 4.3D), but phages did still show a significant increase in infectivity in the direction of dispersal (host population: $F_{1,11} = 1.55$, $P = 0.239$; position: $F_{2,11} = 8.97$, $P < 0.01$; interaction: $F_{2,11} = 0.06$, $P = 0.940$).

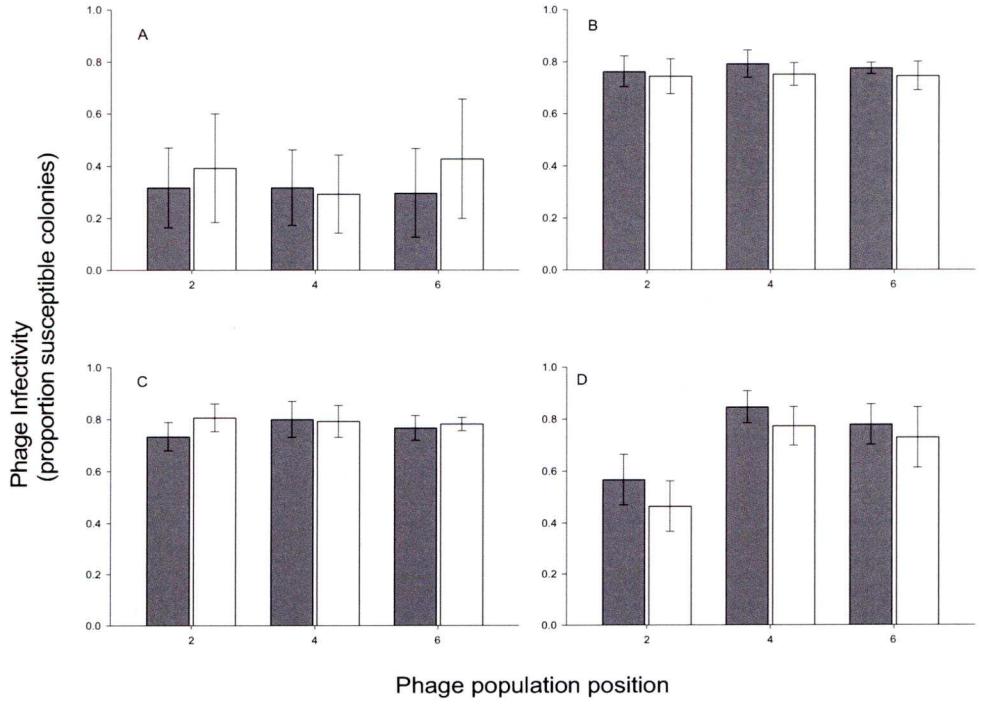

Figure 4.3: Home versus away infectivity. Infectivity (proportion susceptible colonies ± standard error) of phages from positions 2,4 and 6 against home hosts (grey bars) and away hosts (white bars). Panel (A) no dispersal; (B) global dispersal; (C) circular dispersal; (D) linear dispersal.

4.5 Discussion

Patterns of phage infectivity and bacterial resistance ranges were markedly different for isolated landscapes when compared to the dispersal treatments: dispersal consistently led to the evolution of broader phage infectivity ranges relative to bacterial resistance ranges (figure 1). This supports previous work using this system which suggested that phages benefit more from dispersal than their bacterial hosts, due to their lower within-population evolutionary potential in the absence of migration (Morgan et al. 2005; Morgan et al. 2007). Moreover, within linear landscapes infectivity was found to increase with the direction of dispersal, suggesting that dispersal further increased the evolutionary potential of phage populations with each 'step' along the landscape. Phage local adaptation and phage local maladaptation were both detected within the same experimental landscape, but only for linear dispersal networks. Previous work has shown that SBW25Φ2 is unlikely to show local adaptation during the early, directional stages of coevolution (Morgan et al. 2005; Morgan and Buckling 2006), but no previous work had examined landscapes which incorporated spatial variation in infectivity.

Significant parasite local adaptation or local maladaptation was only detected using the local versus foreign definition. This is therefore unlikely to reflect 'true' parasite local adaptation in the sense of a metapopulation-wide increase or decrease in adaptation to local host defences. It is more likely to be reflective of the within-landscape spatial variation in phage fitness and the lack of variation in host fitness. As the local versus foreign definition of local adaptation compares the performance of local and foreign phages against a common host populations, the lack of quantitative variance in host performance causes all variation in local adaptation to be a result of the spatial variation in phage infectivity. For example, parasites with high local infectivity will also possess high global infectivity, and appear locally adapted when compared to parasites with lower infectivity from 'foreign' locations. In contrast, if host resistance did spatially vary but phage infectivity did not, it is likely that the home versus away definition of local adaptation would produce both significant local adaptation and local maladaptation. For example, parasites whose local hosts possess higher global resistance will appear locally maladapted, due to improved parasite performance

on less resistant hosts from 'away' locations. As such, both conventionally used definitions of local adaption will only give congruent results where neither infectivity nor resistance shows significant spatial variation (Thrall et al. 2002).

Although neither of the commonly used definitions of parasite local adaptation will always detect 'true' local adaptation, they will still be measures of the coevolutionary dynamics at a landscape or metapopulation level, particular if coevolutionary traits vary geographically. Geographic variation in the strength of coevolutionary interaction has been reported for a wide range of systems (Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thrall et al. 2002; Benkman et al. 2003; Thompson 2005; Laine 2006; Toju and Sota 2006; Hanifin et al. 2008). These situations will likely result in a mix of locally adapted and local maladapted populations: populations whose geographic location results in greater selection for coevolutionary traits will tend to show local adaptation, whilst those populations experiencing ecological conditions not favouring escalated infectivity and defence traits will be locally maladapted.

As with similar work using bacteria and phage, the experiment reported here migrated bacteria and phage at the same rate, and the results may be more relevant to host-parasite systems where host and parasite display similar degrees of population structuring (Forde et al. 2004; Forde et al. 2007; Morgan et al. 2007). Similarly, dispersal only occurred at one rate in this experiment (1% of founding population at each transfer), which previous work on this system suggests is an intermediate level of dispersal (Morgan et al. 2005; Morgan et al. 2007; Vogwill et al. 2008). Local adaptation is predicted to be strongest at intermediate levels of dispersal (Gandon 2002; Gandon and Michalakis 2002); low rates of dispersal impair adaptation by constraining genetic diversity, while high rates prevent local adaptation by swamping local conditions.

Both theoretical work and empirical studies suggest that the degree of dispersal has a major affect on both host-parasite coevolution and parasite local adaptation. However the findings presented here also demonstrated that spatial dispersal networks can be major drivers of coevolutionary dynamics. Specifically, we have shown that geographic variation in infectivity, as generated by unidirectional

dispersal can drive the evolution of parasite local adaptation and local maladaptation, in the absence of other ecological differences between patches (Morgan et al. 2005; Morgan and Buckling 2006). This demonstrates that spatial dispersal networks can have a major effect on host-parasite coevolution, and provides further support to the notion that host-parasite interactions can only be understood in a spatially explicit context.

Chapter 5: Source populations act as coevolutionary pacemakers in experimental selection mosaics containing hotspots and coldspots

5.1 Abstract

Natural populations of hosts and their enemies are often spatially structured with patches that vary in the strength of reciprocal selection, so-called coevolutionary hotspots and coldspots with strong or weak reciprocal selection respectively. Theory predicts that dispersal from hotspots should intensify coevolution in coldspots, whereas dispersal from coldspots should weaken coevolution in hotspots, however there have been few empirical tests. We addressed this using paired populations of the bacterium *Pseudomonas fluorescens* and the phage SBW25Φ2 linked by one-way dispersal. Within each population, the strength of reciprocal selection was manipulated by altering the bacteria-phage encounter rate, which changes the rate of coevolution without affecting environmental productivity. We observed that dispersal from hotspots accelerated coevolution in coldspots, while dispersal from coldspots decelerated coevolution in hotspots. These results confirm theoretical predictions and suggest that source populations can act as coevolutionary "pacemakers" for recipient populations, overriding local conditions.

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5.2 Introduction

Antagonistic coevolution, the process of reciprocal selection for defence and counter-defence between hosts and their enemies, is pervasive in biological communities and thought to have a wide range of ecological and evolutionary consequences, including driving population dynamics (Thompson 1998; Loeuille et al. 2002; Yoshida et al. 2007), the evolution of diversity (Frank 1993; Benkman 1999; Schluter 2000; Buckling and Hodgson 2007) and the evolution of parasite virulence (Bull 1994; Gandon et al. 2002; Woolhouse et al. 2002). Coevolving populations of hosts and their enemies are often spatially structured, occurring as a set of patches connected by dispersal. The Geographic Mosaic Theory posits that variation in ecological conditions between patches can lead to differences in local selection, generating mosaics in adaptation (Thompson 2005). This can potentially lead to variation in the strength of reciprocal selection between hosts and parasites in different patches, such that some patches display reciprocal selection (hotspots), while others do not (coldspots) (Gomulkiewicz et al. 2000). Dispersal and gene flow between these patches can then act to redistribute genotypes and alleles across the selection mosaic (Thompson 1999, 2005).

A key theoretical prediction is that coevolutionary hotspots need not be ubiquitous to have an effect on the evolutionary dynamics of an interaction across the selection mosaic as a whole (Thompson 2005). Specifically, coevolutionary hotspots can drive coevolution in coldspots provided there is gene flow and sufficiently strong selection within the hotspot (Gomulkiewicz et al. 2000). However, coldspots can also influence evolutionary dynamics in hotspots under certain conditions. For example, when hotspots are surrounded by coldspots, gene flow can lead to the swamping of the hotspot with coldspot-adapted genotypes, which can override local conditions by weakening the response to reciprocal selection pressures (Nuismer et al. 2003). Taken together, these findings lead to the theoretical prediction that dispersal from hotspot to coldspot should intensify coevolution in the coldspot, whereas dispersal from coldspot to hotspot should weaken coevolution in the hotspot.

Geographic variation in the strength of reciprocal selection has been inferred in a number of natural host-enemy systems (Benkman 1999; Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thompson and Cunningham 2002; Thrall and Burdon 2003; Thompson 2005; Laine 2006; Toju and Sota 2006; Hanifin et al. 2008). Indeed much empirical data suggest that classification into coevolving hotspots and non-coevolving coldspots may be rather too simplistic (Nash 2008), and that there is often likely to be a continuum of intensity of reciprocal selection strength between pure coldspots and extreme hotspots (Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thrall and Burdon 2003; Toju 2008). A number of ecological factors have been suggested to cause variation in reciprocal selection pressures, these include abiotic factors such as environmental productivity (Hochberg and Baalen 1998; Lopez-Pascua and Buckling 2008) and climate (Toju and Sota 2006; Toju 2008), and biotic factors such as host-enemy encounter rates (Laine 2006) and the presence / absence of other interacting species (Benkman et al. 2001; Thrall et al. 2007) . However, while geographic variation in reciprocal selection appears to be widespread in natural populations and its importance is highlighted by theory (Thompson 2005), there have been few explicit empirical tests of its impact on coevolutionary dynamics in selection mosaics connected by dispersal.

One reason for this lack of direct empirical data is that controlled, replicated coevolution experiments are extremely difficult to conduct in natural populations where the spatial and temporal scales are large, and rates of dispersal and historical relationships between patches are difficult to determine and control. For these reasons, laboratory populations of bacteria and their viral parasites, phage, have emerged as key model systems for testing aspects of the Geographic Mosaic Theory (Forde et al. 2004; Morgan and Buckling 2006; Brockhurst et al. 2007a; Forde et al. 2007; Morgan et al. 2007; Lopez-Pascua and Buckling 2008; Vogwill et al. 2008). The bacterium *Pseudomonas fluorescens* SBW25 and its naturally associated phage SBW25Φ2 have been used extensively to test coevolutionary theory (Brockhurst et al. 2007b). Persistent arms-race coevolution with directional selection for increased bacterial resistance and phage infectivity range has been observed, suggesting a multilocus gene-for-gene interaction (Buckling and Rainey 2002; Poullain et al. 2008). Crucially, because population samples can be cryogenically stored in "suspended animation", it is possible to directly measure

rates of coevolutionary change through time. Increasing within-population mixing, by periodically shaking culture vessels, has been shown to increase the strength of reciprocal selection by raising the bacteria–phage encounter rate; this strengthens selection for resistance and by extension for novel infectivity thereby accelerating coevolution, approximately doubling its rate, but has no effect on environmental productivity (Brockhurst et al. 2003). Here, we use this simple environmental manipulation to create patches within experimental selection mosaics that vary in the strength of reciprocal selection (strong reciprocal selection / with population mixing, henceforth "PM^+"; weak reciprocal selection / without population mixing, henceforth "PM^-").

Experimental landscapes each consisted of two populations of *P. fluorescens* and SBW25Φ2 connected by unidirectional dispersal such that one population acted as a source of migrants and the other as a recipient of migrants. Four possible source-recipient arrangements were investigated: [1] PM^- source-PM^- recipient, [2] PM^+ source-PM^+ recipient, [3] PM^- source-PM^+ recipient and [4] PM^+ source-PM^- recipient. Arrangements 1 and 2 represent homogeneous landscapes, while 3 and 4 are heterogeneous with regard to population mixing and therefore strength of reciprocal selection. In addition, two rates of between population dispersal were investigated. Populations were propagated by batch culture for a total of twelve transfers, and every two transfers the rate of coevolution in each recipient population was measured.

Theory predicts that the influence of hotspots on coldspots is likely to be dependent on the rate of dispersal, with intermediate rates of dispersal required for hotspots to have any effect (Gomulkiewicz et al. 2000). To test this, a second experiment was performed utilising a wider range of dispersal rates. Specifically, we established another set of PM^+ source-PM^- recipient and PM^- source-PM^- recipient landscapes which were exposed to four rates of dispersal. To track the movement of host from sources to recipients during this experiment, within each landscape either the source or recipient population was founded using *lacZ* marked strain of *P. fluorescens* (Zhang and Rainey 2007), while its partner population contained ancestral *P. fluorescens*. As in the previous experiment, landscapes were propagated for twelve transfers, and every two transfers assays

were performed on the rate of coevolution and the extent of host invasion from source to recipient population.

5.3 Materials and Methods

5.3.1 Culturing Techniques

Populations were propagated by batch culture in 30mL glass universal bottles with loose fitting plastic caps containing 6 mL of standard King's B medium (KB) in an incubator at 28°C. PM^- populations were incubated statically, PM^+ populations were shaken for one minute in every thirty minutes at 200rpm (Brockhurst et al. 2003). A 60μL aliquot of each population was transferred to fresh media every 48 hours. Samples of culture were stored at -80°C in 20% glycerol. Phage populations were isolated by centrifuging samples of culture in 10% chloroform (which lysed and pelleted bacterial debris) and then stored at 4°C.

5.3.2 Experimental 1: Dispersal between hotspots and coldspots

48 replicate populations (24 PM^- populations and 24 PM^+ populations) were founded with 10^5 clonal particles of phage and 10^8 *P. fluorescens* SBW25 cells, and allowed to coevolve for six transfers prior to beginning dispersal treatments. After this period, populations were assigned into source-recipient pairs to create six replicates of each of the following source-recipient arrangements: [1] PM^- source-PM^- recipient, [2] PM^+ source-PM^+ recipient, [3] PM^- source-PM^+ recipient and [4] PM^+ source-PM^- recipient. Each source-recipient pair was used to found two experimental landscapes, one to undergo 1% dispersal and one to undergo 10% dispersal. 6 PM^+ and 6 PM^- recipient populations were also used to found isolated populations that received no migrants. 60μL aliquots were transferred to fresh microcosms every 48 hours for a total of twelve transfers. Source to recipient population dispersal was achieved by, for each recipient population, a defined portion of this transferred aliquot being contributed by the corresponding source population. Depending on the dispersal rate this involved

either transferring 54μL of recipient population and 6μL of source population to a fresh microcosm (10% dispersal rate), or 59.4μL of recipient population and 0.6μL of source population (1% dispersal rate).

5.3.3 Experiment 2: Interactions with dispersal rate

18 replicate microcosms were established containing 10^7 cells of *SBW25* and 10^5 particles *SBW25Φ2* and allowed to diverge for six experimental transfers to generate divergence between populations. Of these, six populations are referred to as recipients and propagated in a static incubator; another six were designated as PM^- sources and propagated in a static incubator; while the final six were designated as PM^+ sources and propagated in a shaken incubator. Within each set of six, half were founded with unlabelled *SBW25* and half with X-gal marked *SBW25*.

Following divergence, recipient populations were each used to found 9 replicate populations, while source populations were each used to found 4 replicates. One set of recipient replicates was maintained in the absence of migration, while each set of source replicates was paired with one of the remaining sets of recipient populations, creating eight experimental landscapes of six populations each. Half of these landscapes contained PM^+ sources while half contained PM^- sources. Within each landscape, 3 source-recipient pairs consisted of X-gal marked populations as recipients and un-marked populations as sources, while the other three source-recipient pairs contained the opposite combination, thereby allowing the progress of any host invasions from source to sink to be tracked.

Landscapes were then propagated for twelve further experimental transfers. At each transfer, 1% of each source population was transferred to a fresh microcosm. Recipient populations also received the same amount of transfer (1%), but some of this culture also came from the corresponding source population. Four migration rates between source and recipient were used: 10%, 1%, 0.1% and 0.01% of total inoculum, as well as 0% controls. One set of the replicate

experimental landscape containing PM^+ source populations and one set containing PM^- source populations was subjected to each migration rate.

5.3.4 Measuring Coevolution

Bacterial resistance for a given population was determined by isolating 10 bacterial colonies on KB agar, which were then streaked across a perpendicular line of phage that had been previously dried onto a KB agar plate. Any bacterial colonies that showed growth inhibition upon encountering the line of phage were classed as sensitive. Resistance was measured as the proportion of resistant bacterial colonies. Antagonistic coevolution between *P. fluorescens* and SBW25Φ2 has been shown to be predominantly escalatory with directional selection for increasing infectivity and resistance through time (Buckling and Rainey 2002; Brockhurst et al. 2003). To determine the rate of coevolution, we measured how the infectivity of phage populations to a bacterial population changed through time. Specifically, every two transfers we determined the resistance of bacterial populations to past (two transfers previous) and future (two transfers subsequent) sympatric phage populations. If directional coevolution was occurring then we would expect, for multiple time points, future phage to be better than past phage at infecting contemporary bacteria, hence a positive slope of infectivity against time: the magnitude of this slope gives a measure of the rate of coevolutionary change .

5.3.5 Tracking Bacterial Invasions

To monitor proportions of *SBW25* and *SBW25lacZ,* recipient populations from experiment 2 were plated every two transfers on KB agar which had been enhanced with 40µl of 0.1M isopropyl thiogalactoside (IPTG) and 40µl of 20mg/ml x-gal. IPTG increases the rate of transcription of the *lacZ* operon, which encodes β-galactosidase that catalyses x-gal and produces a blue colour, allowing for the blue/white screening of colonies.

5.3.6 Statistical Analysis

For experiment 1, rates of coevolution were averaged through time and analysed using a linear mixed model. Source population mixing, recipient population mixing, and dispersal rate were fitted as fixed factors, while founding population was fitted as a random factor nested within both source and recipient population-mixing.

Similarly, for experiment 2, rates of coevolution were also averaged through time and analysed using a linear mixed. Source population mixing was treated as a fixed factor, and log10 of dispersal rate +0.01 was fitted as a covariate in a fully-factorial design. Founding populations and strain *(SBW25 or SBW25lacZ)* were fitted as random factors.

The proportion of invasive host strains was analysed by also averaging across all time points and analysed using a linear mixed model. Dispersal rate, bacterial strain and source mixing rate were treated as fixed factors in a fully factorial design, and founding population was treated as a random factor.

5.4 Results

5.4.1 Experiment 1

In the absence of immigration, population mixing had a significant effect on the strength of reciprocal selection within populations ($F_{1,10} = 12.62$, $P < 0.01$), confirming that the PM^+ treatment created hotspots (mean rate of coevolution = 0.312 ± 0.016) while the PM^- treatment created coldspots (mean rate of coevolution = 0.226 ± 0.018). Within experimental landscapes, the coevolutionary rate of recipient populations was determined by population mixing in the source population (Figure 5.1; $F_{1,20} = 4.503$, $P = 0.047$), but not by population mixing in the recipient population itself (Figure 5.1; $F_{1,20} = 0.328$, $P = 0.573$), nor by the rate of immigration (Figure 5.1; $F_{1,20} = 0.840$, $P = 0.370$), and there were no significant interactions between the main-effects (Table 5.1). Therefore as

predicted, immigration from PM^+ source populations increased the rate of coevolution in PM^- recipient populations, while immigration from PM^- source populations decreased the rate of coevolution in PM^+ recipient populations, relative to equivalent recipient populations in homogeneous landscapes.

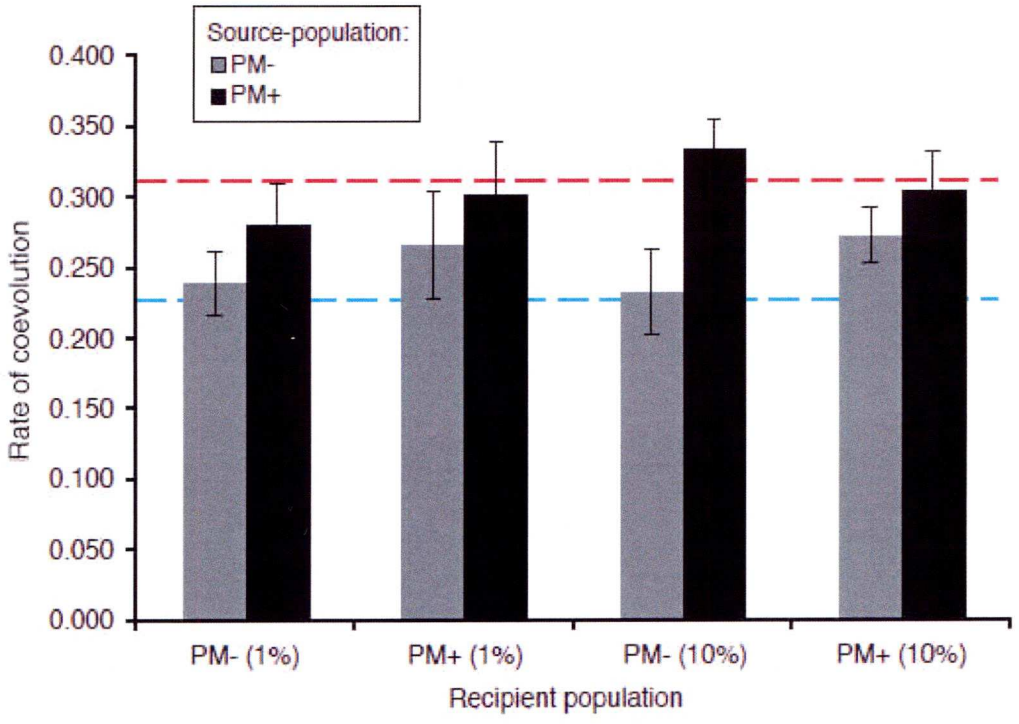

Figure 5.1: Rate of coevolution in recipient populations in Experiment 1: dispersal between hotspots and coldspots.

Bars represent the mean rate of coevolution averaged through time ± SE in recipient populations. Dotted lines represent the mean rate of coevolution in PM^+ (upper line, red) and PM^- (lower line, blue) populations that received no migrants. Source-type refers to the population-mixing regime in the source population, while recipient-type refers to the population-mixing regime in the recipient population. Rates of between patch dispersal are provided in brackets.

Table 5.1: Tests of fixed effects in Experiment 1: dispersal between hotspots and coldspots.

Source	Numerator df	Denominator df	F	p
intercept	1	20	510.825	**<0.001**
SM	1	20	4.503	**<0.05**
RM	1	20	0.328	0.573
DR	1	20	0.840	0.370
SM * RM	1	20	0.600	0.448
SM * DR	1	20	0.840	0.370
RM * DR	1	20	0.352	0.560
SM * RM * DR	1	20	1.049	0.318

Key: SM – source mixing rate; RM – recipient mixing rate; DR – dispersal rate.

5.4.2 Experiment 2

Rate of coevolution in recipient populations (figure 2) was again found to be determined by the rate of mixing in source populations ($F_{1,39} = 5.62$, $P < 0.05$), and again was not significantly affected by the rate of dispersal between source and recipient populations ($F_{1,39} = 1.79$, $P = 0.189$), nor any interaction between rate of source mixing and dispersal rate ($F_{1,39} = 0.43$, $P = 0.515$).

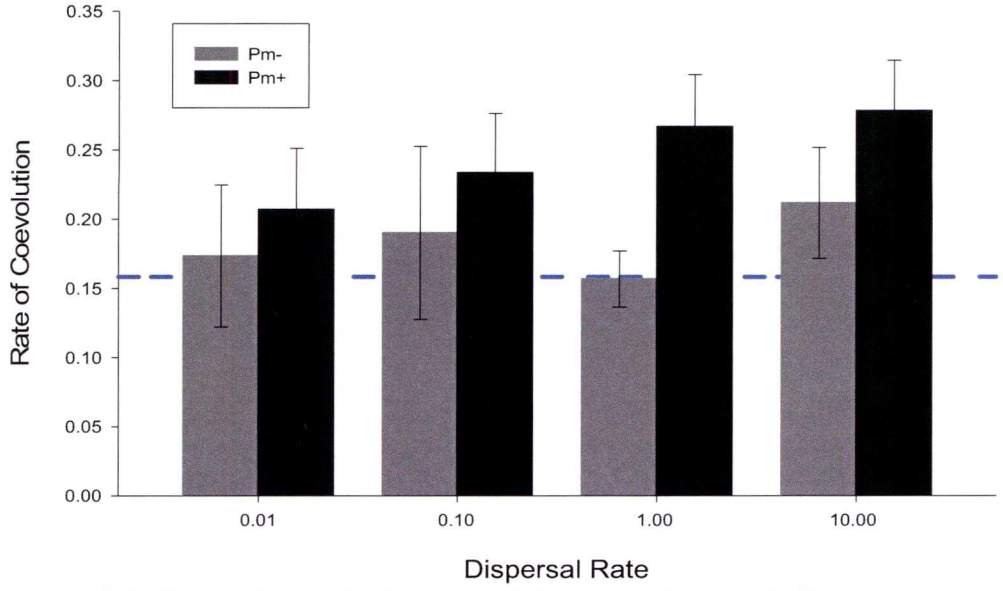

Figure 5.2: Rate of coevolution in recipient populations in Experiment 2: Rate of dispersal from hotspots.

Bars represent the mean rate of coevolution averaged through time ± SE in recipient populations connected to PM- sources (grey bars) and PM+ sources (black bars). The dotted line represent the mean rate of coevolution in PM^- (blue) populations that received no migrants.

Collection of data about host invasions was discontinued after only two points (transfers 10 and 12) had been collected (table 5.2). This was due to *SBW25lacZ* being at a consistently lower density across all treatments, indicating a previously unreported metabolic cost of the inserted *lacZ* operon. Specifically, the proportion of bacteria originating from the source population was found to be unaffected by dispersal rate (table 2; $F_{3,28} = 0.25$, $P = 863$), but strongly affected by the identity of the invasive strain (table 2; $F_{1,4} = 17.27$, $P < 0.05$). Specifically, the proportion of *SBW25lacZ* was lower in populations linked to PM^+ source populations than those linked to PM^- source populations (table 2; $F_{1,28} = 22.83$, $P <0.001$), indicating a genotype by environment interaction whereby the *lacZ* operon is increasingly costly with increasing rate of coevolution. All other interactions were non-significant (table 5..3).

Table 5.2: Proportion of Bacteria in Recipient Population originating in Source Population ± SE

	Dispersal Rate	10	1	0.1	0.01
Source Mixing	**Source Strain**				
Pm+	**SBW25lacZ**	0.03 ± 0.03	0.04 ± 0.02	0.14 ± 0.11	0.00 ± 0.00
Pm+	**SBW25**	0.91 ± 0.08	1.00 ± 0.00	0.90 ± 0.10	0.95 ± 0.05
Pm-	**SBW25lacZ**	0.33 ± 0.25	0.41 ± 0.27	0.27 ± 0.14	0.29 ± 0.15
Pm-	**SBW25**	0.70 ± 0.19	0.60 ± 0.21	0.66 ± 0.17	0.52 ± 0.25

Table 5.3: Tests of fixed effects on proportion of bacteria in recipient population originating in source population

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	4	46.657	**<0.005**
DR	3	28	0.246	0.863
SM	1	28	0.178	0.677
HS	1	4	17.267	**<0.05**
DR * SM	3	28	0.167	0.918
DR * HS	3	28	0.04	0.989
SM * HS	1	28	22.826	**<.001**
DR * SM * HS	3	28	0.555	0.649

Key: HS – host strain; DR – dispersal rate; SM – source mixing.

5.5 Discussion

Central to the Geographic Mosaic Theory is the concept of selection mosaics with patches that vary in intensity of reciprocal selection, so-called coevolutionary hotspots and coldspots (Thompson 2005). Such geographic variation in reciprocal selection intensity appears to be widespread in natural host-enemy populations

(Benkman 1999; Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thompson and Cunningham 2002; Thrall and Burdon 2003; Thompson 2005; Laine 2006; Toju and Sota 2006; Hanifin et al. 2008). In this study we experimentally manipulated the strength of reciprocal selection within populations through altering host-parasite encounter rates without affecting environmental productivity. Our results suggest that heterogeneity in the strength of reciprocal selection across a landscape is an important determinant of coevolutionary dynamics within population patches. Specifically, for recipient populations in heterogeneous landscapes, immigration from a patch with stronger reciprocal selection can accelerate coevolution, while immigration from a patch with weaker reciprocal selection can decelerate coevolution. This suggests that source-populations can act as coevolutionary "pacemakers" for recipient-populations, overriding local conditions.

The data presented in figure 5.1 may be seen to question whether dispersal enhances coevolution in the absence of selection mosaics. Figure 5.1 was generated using a time-shift assay, which measures the rate of coevolution in terms of the relative frequency of changes in infectivity and resistance. Its precision is therefore somewhat limited by the stochastic nature of when changes in resistance and infectivity actually occur, and is unlikely to detect fine scale variations in the rate of coevolution. Furthermore, a time-shift assay does not measure the size of these changes, and Morgan et al (2008) has previously shown that simultaneous migration of hosts and parasites does increase the breadth of both host resistance range and phage infectivity range. As such, although figure 5.1 suggests that simultaneous migration does not have a major effect on how often a coevolutionary change occurs, it is likely to still be enhancing coevolution by selecting for changes of larger size.

In experiment 1, it is notable that only low to moderate rates of dispersal were required to override local selection: as little as 1% immigration every ~7.5 host generations. Furthermore, no significant effect of rate was again detected in experiment 2, even with as little 0.01% immigration, although there are obvious caveats when interpreting this experiment (see below). Specifically, theory predicts that coevolutionary dynamics in coldspots should be more likely to

resemble those in hotspots as the migration rate increases from low to moderate levels (Gomulkiewicz et al. 2000). It is possible that the two rates of dispersal (1% and 10%) used in experiment were too similar to detect a significant difference, both being in effect moderate rates of dispersal, and that an even lower dispersal rate would be required to detect the pattern predicted by theory. It is interesting to note that another recent study into the effects of dispersal rate on adaptation also found little difference between the effects of 1% and 10% dispersal (Venail et al. 2008).

It is unclear whether the results of experiment 2 can really be interpreted as evidence for dispersal rate having no effect in coevolutionary dynamics due to *SBW25lacZ* bacteria unexpectedly suffering from reduced competitive ability in the presence of phages. Previously, the *lacZ* operon has been shown not to be significantly costly in both laboratory and field conditions (Zhang and Rainey 2007). However, genotype by environment interactions are relative common for genetically modified microbes (De Leij et al. 1998). Benign laboratory environments are generally nutrient rich enough to compensate for any costs associated with genetic transformation, while conversely more costly environments do reveal costs (De Leij et al. 1998). Previously, coevolving with phages has been shown to be costly to *SBW25* (Brockhurst et al. 2004) and that the relative size of this cost is increased by epistatic interactions with deleterious mutations (Buckling et al. 2006). The reduction in *SBW25lacZ* density in PM^+ linked populations suggests that the cost of the insertion of the *lacZ* operon increased with the accelerated coevolution (i.e. a genotype by environment interaction). Conversely *SBW25* has been shown to suffer no significant reduction in density associated with accelerated coevolution as a result of population mixing (Brockhurst et al. 2003).

In spite of this, significant heating of recipient populations was still detected in experiment 2, suggesting phage dispersal alone may be capable of driving accelerated coevolution. Similar results have previously been reported for this system (Morgan et al. 2005; Vogwill et al. 2008); in the absence of migration, bacteria are ahead in the coevolutionary arms-race due to their greater evolutionary potential (Buckling and Rainey 2002), but dispersal can reverse this

trend (Morgan et al. 2005). This is likely due to a bacterial population containing 'unused' evolutionary potential for resistance evolution (Morgan et al. 2007), for which there is currently no selective benefit due to the relatively slower rate of phage adaptation. However, we cannot explicitly rule out an influence of simultaneous bacteria-bacteriophage migration that is different to purely phage migration, and this will be investigated in future experiments where bacteria and phage dispersal rates will be independently manipulated.

Hosts and parasites were co-dispersed at equal rates in our experiment, while in some host-parasite associations such congruent patterns of host and parasite gene flow are observed (Mulvey et al. 1991), in certain others, patterns of host and parasite gene flow are decoupled with either the host (Delmotte et al. 1999) or the parasite (Dybdahl and Lively 1996; Davies et al. 1999) displaying relatively greater levels of gene flow. As in previous studies (Forde et al. 2004; Forde et al. 2007; Morgan et al. 2007) our findings may therefore be somewhat limited to host-parasite systems that experience simultaneous host-parasite dispersal. Such situations are likely to arise where the parasite is reliant upon the host for its dispersal, as is the case for contact transmitted parasites, or where co-dispersal of host and parasite is driven by an external factor such as a prevailing wind or aquatic current.

These results confirm, along with the findings of a previous experimental study that manipulated environmental productivity (Forde et al. 2007), that dispersal from hotspots can "warm-up" coevolution in coldspots. However, ours is the first, as far as we are aware, to show empirically that dispersal from populations with weaker reciprocal selection can "cool-down" those with more intense reciprocal selection. This has been shown to be theoretically possible (Hochberg and Baalen 1998; Nuismer et al. 2003; Thompson 2005), however, it is important to consider whether hotspots or coldspots are likely predominate in natural selection mosaics. In selection mosaics generated by productivity gradients, where there is likely to be a positive relationship between productivity and population density (Lopez-Pascua and Buckling 2008), it is probable that hotspots will have a greater impact because they will act as net sources of migrants, while coldspots will act as net recipients. However, where reciprocal selection is weakened through reduced

host-parasite encounter rate (Laine 2006), such coldspot populations may act as net sources of migrants due to lower incidence of parasitism, which can negatively regulate host population growth in nature. In addition, geographical limitations to dispersal may often result in unidirectional movement of migrants (e.g., aquatic currents, prevailing winds etc); under such conditions coldspots or hotspots acting as net sources of migrants are likely to determine coevolutionary dynamics across the selection mosaic.

Chapter 6: General Discussion

6.1 Summary

The overall take-home message from this thesis is that introducing spatial structure radically alters both the ecology and evolution of host-parasite systems. Chapter 2 demonstrates that the impact of coevolving parasites on a host's demography depends on the level of spatial structuring in the system, while the impact of spatial structuring on a host's demography is dependent on the presence or absence of a coevolving natural enemy. This was driven by phages causing deterministic cycling of host populations, which were not observed in the absence of phages, and became phase-locked by dispersal. This along with another recent study (Vasseur and Fox 2009) confirms a longstanding theoretical prediction that cyclical systems are more prone to syncronisation by dispersal than noncyclical systems (Bjornstad et al. 1999).

It is currently unknown whether host-parasite demographic interactions or host-parasite coevolutionary interactions are the major determinants of the observed patterns in chapter 2. This could be experimentally investigated by determining if rapid rates of phage infectivity evolution correlate with low bacterial densities (Buckling and Hodgson 2007), or conversely if periods of phage infectivity stasis correlated with peaks of bacterial density. If this was found to be the case, it would suggest that coevolution was the major driver of the bacterial population dynamics. At the same time, correlations between phage and bacteria population densities could be investigated to test the alternative hypothesis that bacteria-phage demographic interactions are the major drivers of bacterial population dynamics.

Aside from being potential caused by coevolutionary dynamics, the observed population dynamics are likely to have feedback effects on the rate and manner of coevolution. This could be investigated while at the same time as testing the predicted role of genetic diversity as the mechanisms through which dispersal alters adaptation. Specifically, dispersal should increase within-patch diversity but

reduce between-patch diversity. As such, the rate of coevolution at the population level should be highest within the localized dispersal treatment, as it should lead to high levels of within-patch diversity while maintaining some between patch-diversity, as predicted by the levels of population synchrony. Levels of bacterial resistance diversity and phage infectivity diversity could be measured either directly by phenotypic assays, or indirectly by the genetic diversity of bacterial receptor sites or phage tail-fibres. If both approaches were adopted, it could also be used to construct a phenotype-genotype map of this interaction, increasing the range of hypotheses that could be addressed using this system.

Chapters 3 and 4 both highlight that the coevolutionary dynamics of host-parasite systems are altered by the introduction of spatial structuring, but also that the nature of spatial structuring (i.e., the spatial dispersal network) determines how coevolution is specifically altered. Chapter 3 empirically confirms a longstanding theoretical prediction (Gandon 2002) that intermediate rates of dispersal maximise the evolutionary potential of parasites. This is reflected in both the rate of coevolution as demonstrated by a time-shift assays, as well as in the levels of phage infectivity and bacterial resistance. The latter of these is interesting as it is a correlation between one organism's dispersal rate and another organism's phenotype, suggesting the dispersal-adaptation relationship in one species may also cause other ecological phenomena to correlate with dispersal, particularly in coevolutionary interactions.

Chapter 4 highlights that asymmetrical dispersal patterns, where some populations receive a greater diversity of migrants than others, also impacts on coevolution and can create geographic variation in the levels infectivity or resistance. This can have important consequences as it may prevent landscape wide, or 'true,' patterns of local adaptation (Gandon and Nuismer 2009), but equally can create areas of local adaptation and local maladaptation in close proximity to each other. This chapter also suggests that the major drivers of coevolutionary dynamics in some natural systems may well be specific to individual populations, such as its position in a dispersal network or its environmental microclimate, and that these are the systems that are unlikely to show 'true' local adaptation.

Chapter 5 confirms a longstanding tenet of the GMTC that demographic sources within geographic mosaics containing hotspots and coldspots act as pacemakers that drive landscape-wide rates of coevolution, overriding local conditions. These results confirm a previous experimental study that manipulated environmental productivity (Forde et al. 2007) that dispersal from hotspots can accelerate coevolution in coldspots. In addition, they demonstrate that dispersal from coldspots can decelerate coevolution in hotspots. Interestingly, theory often predicts hotspots should be major drivers of coevolution in other populations but that coldspots should have less impact (Thompson 2005). This is often because hotspots are predicted to be caused by areas of high local population productivity (Nuismer et al. 2003). However when hotspots and coldspots are not caused by demographics, this chapter demonstrates that both coldspots and hotspots may exert the same degree of influence.

The Merits of Microbial Experimental Evolution

There are obvious practical benefits to using microbial systems as experimental models; they allow experimental and biological issues to be addressed using real genetic systems that possess far greater complexity than can be generated using mathematical simulations. However, the way in which this is practically performed is often too simplistic, too artificial or too experimentally arbitrary to have any biological realism. Bacteria-bacteriophage systems are less prone to this criticism as the dynamics of the system are still the biological interaction between natural enemies, albeit in an artificial setting. The observed molecular, population and evolutionary dynamics of bacteria and phages in laboratories *should* still be occurring in natural populations, and it would be of obvious advantage if this could be confirmed for wild populations of bacteria and phage. This would also allow the testing of evolutionary hypotheses that are directly relevant to the microbes themselves, as opposed to hypotheses that are theoretical or computer generated. Testing generic evolutionary and ecological theories with no regard for their relevance for microbes can be rightly criticised for being arbitrary and contrived, and there is a need for a greater integration of traditional microbiology with experimental evolution.

6.2 Future Directions

The rate of evolutionary and coevolutionary change was once considered to be too slow to influence ecological dynamics (Slobodkin 1961). However there is increasing evidence that evolutionary changes can occur in ecological time and can be used to explain ecological phenomena (Antonovics 1976; Thompson 1998; Hairston et al. 2005). Such eco-evolutionary dynamics are considered particularly relevant in host-parasite systems (Altizer et al. 2003). If areas of rapidly evolving parasites and pathogens need not be ubiquitous to have major ecological effects (chapter 5), it is of major importance to determine where and why these bursts of rapid evolution occur. The substantial knowledge of how and why microbes rapidly evolve under laboratory conditions (Bohannan and Lenski 2000) can be a useful tool in predicting which aspects of microbial phenotypes are currently under selection in natural populations (Buckling et al. 2009). Due to the major importance of microbes for ecology, health, and agriculture, applying the laboratory knowledge of microbial evolution to real world situations is a major challenge in modern evolutionary microbiology.

As spatial structuring is a major driver coevolution, it is important to incorporate or account for spatial structuring in studies of coevolution in natural environments (Thrall et al. 2002; Thompson 2005). This is particularly relevant for studies of microbial host-natural enemy interactions, as the relevant scales on which microbes are sometimes spatially structured is on the scale of centimetres (Vos et al. 2009). Although microbial ecology is in its infancy (Ash et al. 2008), it has great potential as to tool to study spatially extended coevolution, as well as other ecological and evolutionary processes which occur of broad spatial scales (Ash et al. 2008). In particular, bacteria-phage interactions are a very suitable ecological system for demonstrating all the aspects of a geographic mosaic of coevolution. Field studies of microbes would be a natural complement to the traditional laboratory based experimental evolution: ecological and evolutionary patterns could be identified in wild microbial populations, and the hypothesised mechanisms generating them could be tested in laboratories.

To achieve this, studies of bacteria-phage interactions in natural environments would benefit from a closer integration of phenotypic and genotypic analysis. Current studies have either focussed on genomic culture-independent techniques (Andersson and Banfield 2008; Kunin et al. 2008; Tyson and Banfield 2008), or more traditional culturing-based microbiology (Waterbury and Valois 1993; Bruttin et al. 1997; Vos et al. 2009). The use of rapid sequencing technologies has led to the 'second age' of microbial ecology and a much better understanding of bacterial and phage genomic diversity (Ash et al. 2008). However, integration with culture-based studies is still required to associate phenotypes with genotypes and assess the ecological significance of these detected large volumes of diversity. In particular, studying the genetic dynamics of CRISPR units is a fast-paced and interesting area of research (Heidelberg et al. 2009), but as of yet has not been combined with any culture-based analysis.

This would further be aided by a better understanding of the genetic specificity of the interaction between bacteria and phage. For SBW25Φ2 and SBW25 the relevant genes involved in infection are beginning to be identified (Patterson et al (in submission)). Initially, bacteria-phage interactions were compared with the gene-for-gene systems of plant-pathogen interactions, as both processes led to a mix of generalist and specialist phenotypes (Buckling and Rainey 2002; Poullain et al. 2008). However, as there are several lines of bacterial defence that phages must overcome in order to successfully infect a bacterial cell (chapter 1), specificity is likely governed at multiple hierarchical levels, each of which could potentially be governed by a different form of specificity genetics.

The major limitation of SBW25Φ2 is that it is a lytic virus – successful infection always leads to the death of the host bacteria, and therefore represents a semelparous obligate killing parasite. A broader range of host-parasite interactions could be studied if bacteriophages with alternative life-histories were isolated and utilised. Lysogenic or temperate phages still must eventually lyse the host cell in order to produce daughter particles, but lysis can be delayed (Bull 2006). Chronic phages, sometimes referred to as filamentous phages, are able to reproduce without lysing host cells. Instead, daughter viral particles are released through host membrane protein channels, without necessarily killing the host cell

(Weinbauer 2004). Bacteria are also attacked by a wide range of other natural enemies. For example, *Bdellovibrio sp.* are bacterial parasites of other bacteria – they enter the periplasmic space between the inner and outer membranes of other Gram-negative bacteria, and then feed on the resources of the host cell (Davidov and Jurkevitch 2004). In contrast, numerous protists and bacteria predate bacteria by enveloping whole cells, and several of these predators have begun to be used to study predator-prey interactions (Meyer and Kassen 2007; Hillesland et al. 2009). In natural environments, organisms are attacked by multiple different parasites and predators, and diffuse coevolution between multiple species is likely to be a major driver of ecology and evolution. Incorporating a greater number and a greater variety of the natural enemies of bacteria would permit these sorts of questions to be addressed.

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Appendix 1: Vogwill et al (2009)

ECOLOGY LETTERS

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LETTER

Dispersal and natural enemies interact to drive spatial synchrony and decrease stability in patchy populations

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Abstract

Spatial synchrony is widespread in natural populations but the mechanisms that underpin it are not yet fully understood. Two key biotic drivers of spatial synchrony have been identified: dispersal and trophic interactions (e.g. natural enemies). We used spatially structured, patchy bacterial populations to show that although increased dispersal always enhanced spatial synchrony of fluctuations in bacterial abundance, this effect was far stronger in the presence of a bacteriophage parasite. Bacteriophages drove strong within patch fluctuations in bacterial abundance that became phase locked through dispersal. Furthermore, the way in which stability, measured as constancy, responded to increasing dispersal was qualitatively different depending on whether parasites were present or not. Patch-level constancy decreased with dispersal in the presence of parasites, whereas dispersal increased patch-level constancy in the absence of parasites. Population-level constancy also decreased with dispersal in the presence of parasites, but was unaffected by dispersal in the absence of parasites. These contrasting patterns were likely due to the different role played by dispersal in the presence and absence of parasites, synchronizing dynamics in the former case and averaging stochastic fluctuations in the latter. Taken together, our findings suggest that dispersal and natural enemies can interact to drive spatially synchronous population fluctuations that decrease stability at both the patch and population level.

Keywords

Bacteria, bacteriophage, ecological dynamics, experimental ecology, host–parasite, microcosms, migration, population cycles, spatial structure, trophic interactions.

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INTRODUCTION

Spatial synchrony of ecological dynamics is pervasive in natural populations (Bjornstad *et al.* 1999; Liebhold *et al.* 2004). Dispersal and trophic interactions have emerged as key biotic determinants of spatial synchrony (Liebhold *et al.* 2004). However, the precise way in which each factor affects synchrony, and the consequences for population stability and persistence remain the focus of considerable research (Blasius *et al.* 1999; Holland & Hastings 2008). A robust prediction of a range of theoretical models is that dispersal between patches can synchronize fluctuations that arise from similar driving processes (Bjornstad *et al.* 1999; Liebhold *et al.* 2004). Indeed, comparisons of species that differ in dispersal ability suggest that more dispersive species often display more spatial synchrony (Paradis *et al.* 1999). However, this is not always the case and spatial synchrony may instead be more strongly influenced by climactic factors (Peltonen *et al.* 2002), or may depend on the spatial scale at which synchrony is measured (Sutcliffe *et al.* 1996). Trophic interactions, particularly those with natural enemies, have long been thought to drive population fluctuations (Hanski *et al.* 1993; Krebs *et al.* 1995; Hudson *et al.* 1998). Theory suggests that interaction with a spatially synchronized natural enemy can drive spatial synchrony of the exploited species (Ims & Steen 1990; de Roos *et al.* 1998). Such processes are thought to underpin distribution and abundance patterns in a number of natural systems (Small *et al.* 1993; Ims & Andreassen 2000).

Dispersal and natural enemies are also likely to affect population stability. In this paper, we focus on the constancy component of stability, which measures the

tendency for abundance to remain unchanged through time (Grimm & Wissel 1997). Low constancy implies temporally fluctuating abundance, while high constancy implies temporally constant abundance. Constancy can be measured at the level of an individual patch, giving an estimate of abundance fluctuations at a local scale, or at the whole population level, giving an estimate of abundance fluctuations at a regional scale (Dey & Joshi 2006). Whether patch and population measures of constancy are correlated depends upon the degree of spatial synchrony exhibited by the population.

The effect of dispersal on population stability has been modelled extensively. In general, a non-linear hump-shaped relationship between dispersal rate and population stability is predicted by both single-species (Gyllenberg *et al.* 1993; Hastings 1993) and victim-enemy models (Reeve 1988; Taylor 1990). This arises because, in patchy populations where patch abundances fluctuate asynchronously, recolonization may be insufficient at very low levels of dispersal to counter-balance extinctions of declining patches leading to low population stability. Moderate increases in dispersal may improve population stability because dispersal between out-of-phase patches allows recolonization of declining patches. However, further increases in dispersal are likely to synchronize abundance fluctuations across patches, preventing rescue effects (Brown & Kodric-Brown 1977) and potentially decreasing population stability (Heino *et al.* 1997). Some models predict that the effects of dispersal on population stability are likely to be stronger in the presence of natural enemies (Rohani *et al.* 1996).

Clearly, the effects of dispersal and natural enemies on local and regional dynamics have the potential to interact, resulting in counter-intuitive outcomes on ecological dynamics. A number of studies have tested the effects of dispersal on synchrony and/or stability in spatially structured single-species (Lecomte *et al.* 2004; Dey & Joshi 2006) or victim-enemy populations (Holyoak & Lawler 1996; Holyoak 2000; Ellner *et al.* 2001; Bonsall *et al.* 2002). However, few experimental studies to our knowledge have directly compared effects of dispersal on spatial synchrony and stability in the presence and absence of a natural enemy. There are several likely reasons for this, which include the large spatial and temporal scales involved in studies of natural systems. In addition there are difficulties associated with excluding natural enemies, accurately measuring and manipulating dispersal rates, and controlling for extrinsic variables in nature. However, such difficulties can be overcome by using laboratory populations of fast replicating microbes (Jessup *et al.* 2004; Buckling *et al.* 2009). We propagated replicate 64 patch spatially structured populations of *Pseudomonas fluorescens* SBW25 with and without bacteriophage SBW25Φ2, under three scales of dispersal (global, localized and none). We explored how dispersal and natural enemies interact to affect spatial synchrony of ecological dynamics and population stability.

MATERIALS AND METHODS

Culturing techniques

Each population was propagated in 64 wells on a 96-well microtitre plate (i.e. an eight-well by eight-well grid), each well containing 100 μL of King's B liquid media. Eighteen replicate phage-free spatially structured populations were initiated with approximately 1.7×10^6 bacterial cells per well and 18 replicate phage containing populations were initiated with 1.7×10^6 bacterial cells and 170 viral particles per well using a 96-pin replicator. These densities were chosen to be equivalent to the starting population densities previously used in experiments with this system (Buckling & Rainey 2002a). Populations were propagated by serial transfer for 12 transfers (every 2 days 1 μL of each well was used to inoculate a fresh well using a 96-pin replicator) under one of the following dispersal regimes: global – all patches were pooled and homogenized prior to transfer; localized – the contents of all eight wells in each row were pooled and homogenized prior to even-numbered transfers, the contents of all eight wells in each column were pooled and homogenized prior to odd numbered-transfers; none – no between well mixing occurred prior to transfer.

Analysing synchrony and constancy

After each growth cycle, we measured bacterial abundance in each well as absorbance at 630 nm using an optical density plate-reader (Biotek EL800, Winooski, Vermont, USA). To estimate spatial synchrony we calculated cross correlations at lag-zero of the first differenced time series of log abundance [$\ln(N_t)-\ln(N_{t-1})$, where N_t is the population size at time t] of all patch pairs in the population (Bjornstad *et al.* 1999). To estimate constancy, we calculated the fluctuation index (Dey & Joshi 2006) (FI):

$$\mathrm{FI} = \frac{1}{T\bar{N}} \sum_{t=0}^{T-1} Abs(N_{t+1} - N_t)$$

where $\bar{N}$ is the mean population size over T transfers. FI measures the mean one-step change in abundance, scaled by average population size, over the duration of the experiment. FIs were calculated at both the level of the population and the patch (i.e. individual wells). Levels of population synchrony and FI's at both patch and population levels were analysed using two-way ANOVAs, with presence or absence of phages and level of dispersal fitted as factors. Where significant interactions between phage presence and dispersal were detected, simple effects of phage and dispersal were analysed using orthogonal contrasts, using a Bonferroni corrected alpha value of 0.01.

RESULTS

Bacterial abundance

Population dynamics for representative populations under each experimental treatment are displayed in Fig. 1. Phages and dispersal had interactive effects on bacterial abundance (Fig. 2; phage × dispersal interaction: $F_{2,30} = 3.76$, $P < 0.05$). Phages reduced mean bacterial abundance across all dispersal treatments (Fig. 2; simple effect of phages: no dispersal, $F_{1,30} = 36.1$, $P < 0.001$; localized dispersal, $F_{1,30} = 96.0$, $P < 0.001$; global dispersal, $F_{1,30} = 74.4$, $P < 0.001$) and bacterial abundance decreased with increasing dispersal in the presence of phages (Fig. 2; simple effect of dispersal: $F_{2,30} = 7.65$, $P < 0.01$) but not in the absence of phages (Fig. 2; simple effect of dispersal: $F_{2,30} = 0.09$, $P > 0.01$).

Spatial synchrony

The synchrony of fluctuations in bacterial abundance increased with dispersal for both phage containing (Fig. 3; simple effect of dispersal: $F_{2,30} = 68.9$, $P < 0.001$) and

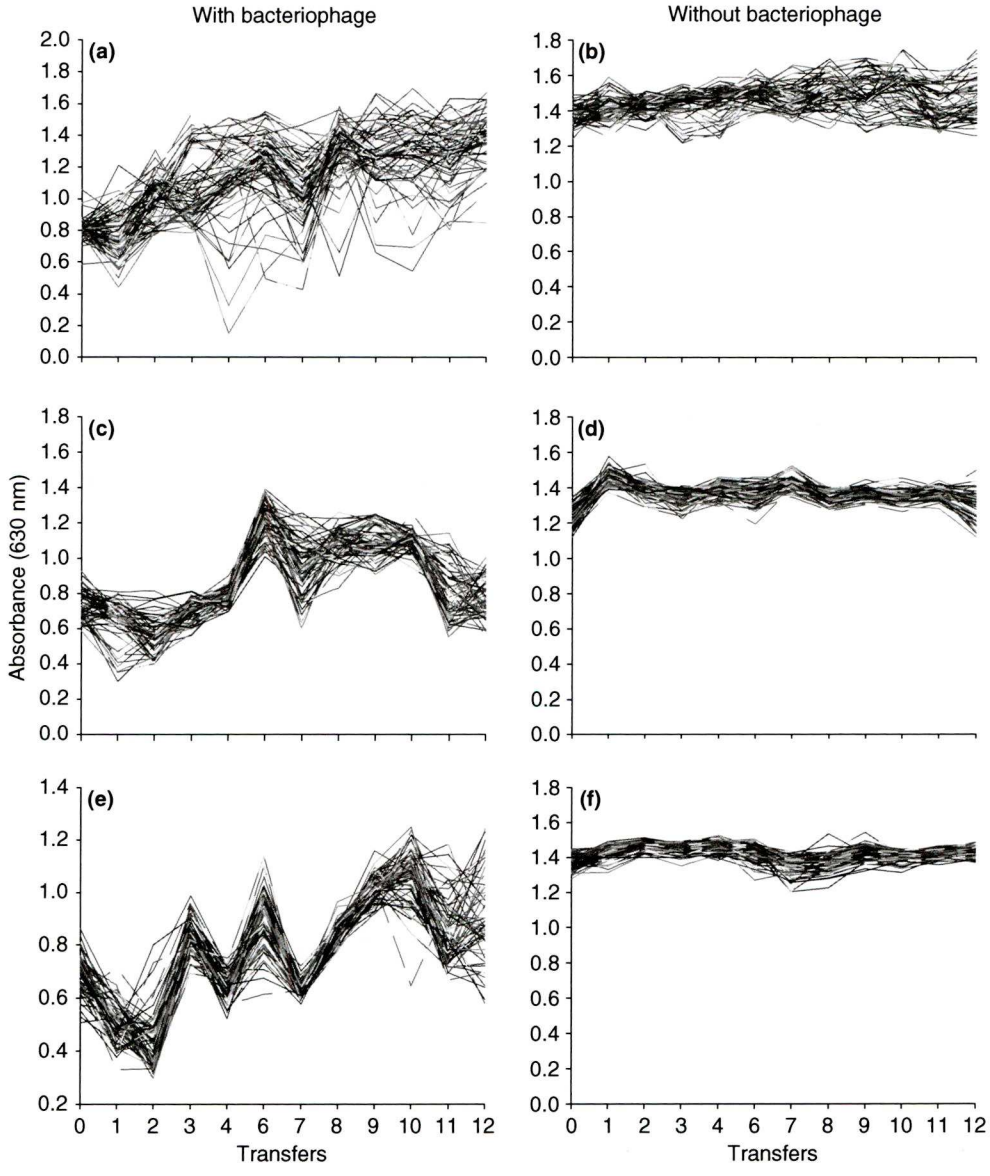

Figure 1 Population dynamics: panels show bacterial abundance dynamics of representative populations under no dispersal (a & b), localized dispersal (c & d) and global dispersal (e & f) in the presence (left-hand panels) and absence (right-hand panels) of bacteriophage parasites. Lines represent bacterial abundance measured as absorbance (630 nm) of individual wells.

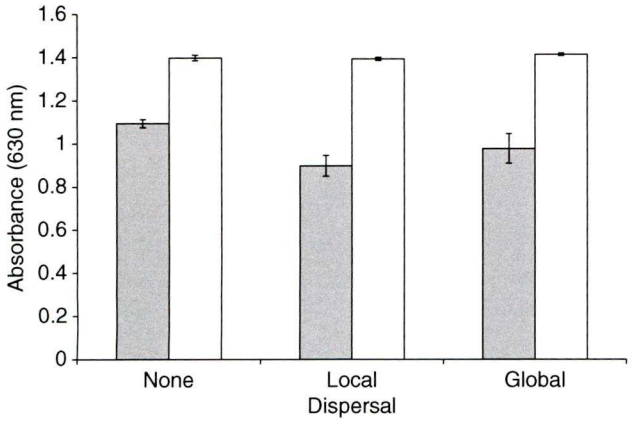

Figure 2 Bacterial abundance: bars represent mean absorbance at 630 nm (± SE) in the presence (grey) and absence (white) of bacteriophage parasites.

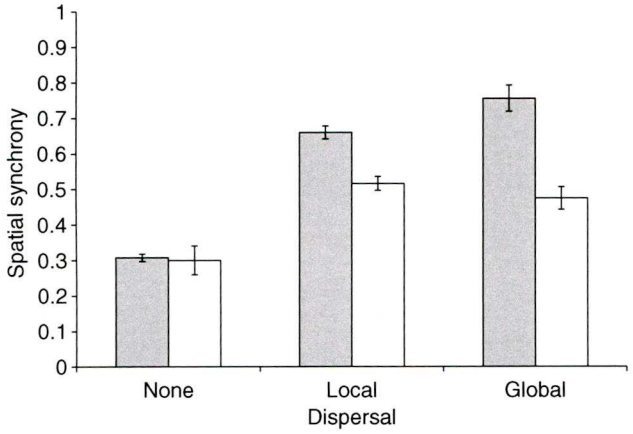

Figure 3 Spatial synchrony: bars represent mean cross correlation coefficients (± SE) in the presence (grey) and absence (white) of bacteriophage parasites.

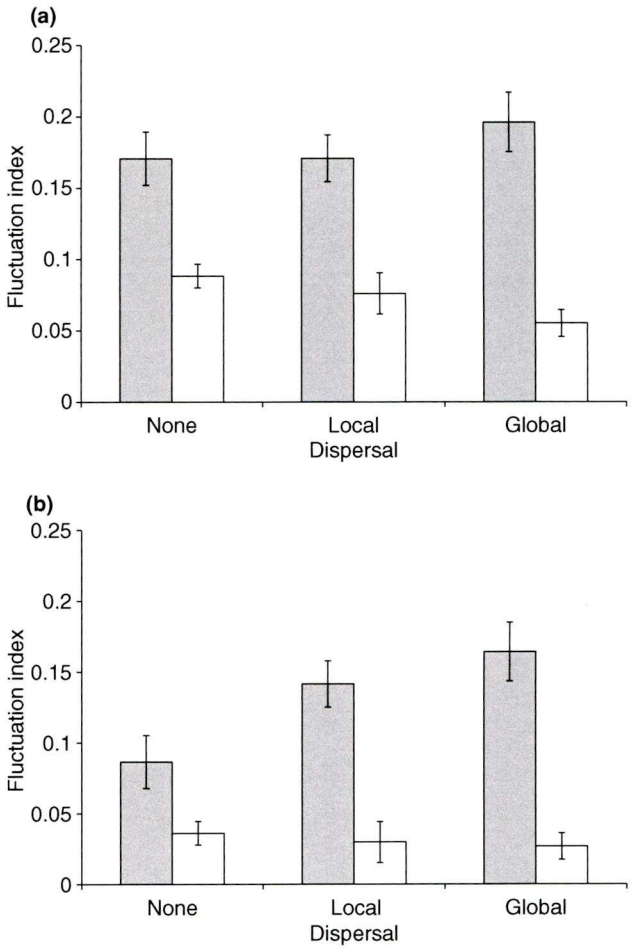

Figure 4 Patch-level and population-level constancy: panels show constancy measured as Fluctuation Index at the patch level (panel a) and population level (panel b). Bars represent mean fluctuation index (± SE) in the presence (grey) and absence (white) of bacteriophage parasites.

phage-free populations (Fig. 3; simple effect of dispersal: $F_{2,30} = 16.2$, $P < 0.001$). However, this effect was far stronger in parasitized compared to unparasitized populations (Fig. 3; phage × dispersal interaction: $F_{2,30} = 11.56$, $P < 0.001$). While phage had no effect on spatial synchrony in populations without dispersal (Fig. 3; simple effect of phages: $F_{1,30} = 0.036$, $P > 0.01$), mean population spatial synchrony was significantly higher in parasitized populations with dispersal (Fig. 3; simple effect of phages: localized dispersal, $F_{1,30} = 12.7$, $P < 0.01$; global dispersal, $F_{1,30} = 48.8$, $P < 0.001$).

Patch-level constancy

Analysis of individual patch dynamics revealed that phages and dispersal had interactive effects on patch-level constancy (Fig. 4a; phage × dispersal interaction: $F_{2,30} = 12.11$, $P < 0.001$). Patch-level constancy was much lower in the presence of phages, which drove strong within patch fluctuations in bacterial abundance (Fig. 4a; simple effect of phages: no dispersal, $F_{1,30} = 113.7$, $P < 0.001$; localized dispersal, $F_{1,30} = 114.3$, $P < 0.001$; global dispersal, $F_{1,30} = 252.6$, $P < 0.001$). In the presence of phages, patch-level constancy decreased with increasing dispersal (Fig. 4a; simple effect of dispersal: $F_{2,30} = 5.45$, $P < 0.01$), while, in contrast, patch-level constancy increased with dispersal in the absence of phages (Fig. 4a; simple effect of dispersal: $F_{2,30} = 7.14$, $P < 0.01$).

Population-level constancy

Dispersal had contrasting effects on population-level constancy in the presence and absence of phages (Fig. 4b; phage × dispersal interaction: $F_{2,30} = 9.79$, $P < 0.001$).

Dispersal in the absence of phages had no effect on population-level constancy (Fig. 4b; simple effect of dispersal: $F_{2,30} = 0.228$, $P > 0.01$), while dispersal in the presence of phages decreased population-level constancy (Fig. 4b; simple effect of dispersal: $F_{2,30} = 15.6$, $P < 0.001$). Overall parasitized populations were much less stable than unparasitized ones (Fig. 4b; simple effect of phages: no dispersal, $F_{1,3} = 54.2$, $P < 0.001$; localized dispersal, $F_{1,30} = 60.9$, $P < 0.001$; global dispersal, $F_{1,30} = 92.3$, $P < 0.001$). This was due to phages driving within-patch fluctuations in bacterial abundance that were synchronized by dispersal, becoming phase-locked across the population as a whole thereby reducing population-level constancy.

DISCUSSION

Our data suggest that the impact of dispersal on local and regional dynamics depends on the presence of natural enemies. These results are likely due to the ways in which stochastic and deterministic fluctuations interact across the population. In the absence of parasites, population fluctuations would have arisen primarily through demographic stochasticity due to random variation in initial densities and growth rates within each patch. The evolution of novel genotypes by *de novo* mutation over the course of the experiment is also likely to have contributed to demographic variation between patches. Dispersal between these randomly fluctuating patches would only have had a moderate synchronizing effect, averaging out stochastic variation. By contrast, the presence of a natural enemy resulted in strong multi-generational, deterministic population cycles that swamped the inherent stochastic fluctuations, such that sub-populations quickly became phase locked in the presence of dispersal. Hence, each patch across the population showed highly synchronized fluctuations diminishing the potential for rescue effects. Indeed, this is in keeping with theory, which predicts that populations with cyclical dynamics should synchronize more strongly through dispersal than those with non-cyclical dynamics (Bjornstad *et al.* 1999).

In addition to the ecological dynamics described so far, it is likely that fluctuations in bacterial abundance in parasitized populations were also driven by coevolution. Previous studies have shown that *P. fluorescens* and bacteriophage SBW25Φ2 undergo rapid antagonistic coevolution with directional selection for increased resistance and infectivity ranges respectively over the timescale of our experiments (Buckling & Rainey 2002a; Brockhurst *et al.* 2007). Such coevolution is known to depress bacterial population density through phage-induced mortality following evolution of phage with broader host range (Buckling & Rainey 2002b; Buckling & Hodgson 2007). The observed fluctuations in bacterial abundance were likely caused in part by evolutionary changes in resistance and infectivity profiles of bacteria and phage, in addition to classical Lokta–Volterra population dynamics. It is increasingly recognized that rapid evolution can affect ecological dynamics in a wide range of systems (Thompson 1998; Hairston *et al.* 2005). Indeed, rapid evolution of resistance in bacteria is likely to have increased persistence by preventing phages from driving bacterial populations extinct.

Hosts and parasites were co-dispersed at equal rates in our experiment. In some host–parasite associations such congruent patterns of host and parasite gene flow are observed (Mulvey *et al.* 1991). However, in certain others, patterns of host and parasite gene flow are decoupled with either the host (Delmotte *et al.* 1999) or the parasite (Dybdahl & Lively 1996; Davies *et al.* 1999) displaying relatively greater levels of gene flow. The potential importance of differential relative rates of dispersal of victims and enemies for ecological dynamics is highlighted by the findings of Huffaker's (1958) classic studies of predator and prey mite species. Here, the greater persistence stability of populations under increased spatial complexity was thought to be due to the greater dispersal ability of prey relative to predatory mites. Our findings may therefore be limited to host–parasite systems that experience simultaneous host–parasite dispersal. Such situations are likely to arise where the parasite relies upon the host for its dispersal, as is the case for contact transmitted parasites, or where co-dispersal of host and parasite is driven by an external factor such as a prevailing wind or aquatic current.

An additional but important caveat of our experiment is that the rates of dispersal we used were relatively high compared to those commonly observed in natural systems (Slatkin 1985). Our dispersal regimes consisted of mass migration events at each transfer, with each growth period between transfers allowing approximately 10 bacterial generations. This equated to Slatkin's m [i.e. proportion of immigrants per patch per generation (Slatkin 1985)] values of $\sim$0.10 for global dispersal and $\sim$0.09 for localized dispersal. Our finding that these relatively high rates of dispersal reduced stability of parasitized populations is in line with theory, which predicts a hump-shaped relationship between dispersal rate and population stability (Taylor 1990). This suggests that lower rates of dispersal may have had a stabilizing effect on ecological dynamics in this host–parasite system. By contrast, in the absence of parasites, the rates of dispersal used did not reduce stability of populations. It is possible that natural enemies altered the range of dispersal rates that led to increased population stability, or alternatively, that dispersal had a weaker impact on ecological dynamics in the absence of natural enemies (Rohani *et al.* 1996).

These results confirm the importance of biotic factors as drivers of spatial synchrony of ecological dynamics.

Dispersal has been shown to increase spatial synchrony in single species (Lecomte *et al.* 2004) and host–enemy systems (Holyoak & Lawler 1996; Holyoak 2000). However, this is the first experimental evidence that dispersal can have qualitatively different effects on ecological dynamics and population stability of a focal species in the presence and absence of a natural enemy. Specifically, while dispersal increased stability in the absence of the parasite, it decreased stability in the presence of parasites by causing spatially synchronized fluctuations in abundance. It is also of note that in this system, natural enemies and dispersal alone were sufficient to drive spatial synchrony without the need for extrinsic forcing (Cattadori *et al.* 2005) as has been predicted by theory (Blasius *et al.* 1999).

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Appendix 2: Vogwill et al (2008)

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The impact of parasite dispersal on antagonistic host–parasite coevolution

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Keywords:

gene flow;
gene-for-gene;
geographic mosaic;
local adaptation;
metapopulation;
migration.

Abstract

Coevolving populations of hosts and parasites are often subdivided into a set of patches connected by dispersal. Higher relative rates of parasite compared with host dispersal are expected to lead to parasite local adaptation. However, we know of no studies that have considered the implications of higher relative rates of parasite dispersal for other aspects of the coevolutionary process, such as the rate of coevolution and extent of evolutionary escalation of resistance and infectivity traits. We investigated the effect of phage dispersal on coevolution in experimental metapopulations of the bacterium *Pseudomonas fluorescens* SBW25 and its viral parasite, phage SBW25Φ2. Both the rate of coevolution and the breadth of evolved infectivity and resistance ranges peaked at intermediate rates of parasite dispersal. These results suggest that parasite dispersal can enhance the evolutionary potential of parasites through provision of novel genetic variation, but that high rates of parasite dispersal can impede the evolution of parasites by homogenizing genetic variation between patches, thereby constraining coevolution.

Introduction

Antagonistic host–parasite coevolution, the reciprocal evolution of enhanced host defence and parasite counter-defence, is pervasive in natural communities and is implicated in a wide range of ecological and evolutionary processes (Thompson, 2005; Woolhouse *et al.*, 2002). Often populations of hosts and parasites are subdivided into a set of patches or demes connected by dispersal (a metapopulation). Under such conditions, the dynamics and outcomes of coevolution are likely to be influenced by the relative levels of dispersal between patches in each of the interacting species. All else being equal it is predicted that the species with the greater level of dispersal will have the upper hand in a given coevolutionary arms race (Gandon & Michalakis, 2002; Greischar & Koskella, 2007; Hoeksema & Forde, 2008). This arises because dispersal introduces novel genetic variation into the population, thereby enhancing its adaptive potential (Lenormand, 2002). However, theoretical and empirical studies suggest that very high levels of dispersal can have a detrimental effect on genetic variation and thereby adaptive potential (Garant *et al.*, 2007). This arises through two mechanisms; first, high rates of dispersal can cause 'genetic swamping' by replacing locally adapted alleles with locally maladapted alleles common in the metapopulation as whole (Alleaume-Benharira *et al.*, 2006); second, high rates of dispersal can homogenize genetic variation among patches thus reducing the supply of novel variation attainable through dispersal (Gandon & Michalakis, 2002). Combined, these processes lead to the prediction that the rate of adaptation is likely to peak at intermediate rates of dispersal.

A wide range of relative rates of gene flow, which is likely to correlate with dispersal rate, have been observed in natural antagonistic associations (encompassing host–parasite and predator–prey). Though some antagonistic associations show remarkably congruent patterns of gene flow (Jerome & Ford, 2002; Mulvey *et al.*, 1991), in others, the rate of gene flow experienced by antagonists are decoupled, with either the host/prey (Delmotte *et al.*, 1999; Martinez *et al.*, 1999) or the parasite/predator displaying greater levels of gene flow (Davies *et al.*, 1999; Dybdahl & Lively, 1996; Michalakis *et al.*, 1994). Relatively greater rates of parasite compared with host dispersal or gene flow are likely to underlie patterns of parasite local adaptation (i.e. greater performance on

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sympatric compared with allopatric hosts) observed in natural populations through provision of genetic variation and thereby enhancement of the adaptive potential of parasites (Dybdahl & Lively, 1996; Gandon & Michalakis, 2002; Greischar & Koskella, 2007; Hoeksema & Forde, 2008; Lively & Dybdahl, 2000). Such local adaptation of organisms causing disease to humans or livestock and crops is of particular concern (Woolhouse *et al.*, 2002), thus an understanding of the coevolutionary impact of greater relative rates of parasite compared with host gene flow is required.

In laboratory studies with bacteria and their viral parasites (phage), where rates of dispersal can be directly manipulated, dispersal between patches has emerged as a key determinant of the outcomes and dynamics of coevolution (Brockhurst *et al.*, 2007a; Forde *et al.*, 2004, 2007; Morgan *et al.*, 2007, 2005). In the *Pseudomonas fluorescens* SBW25–SBW25Φ2 association, bacteria are locally adapted in the absence of dispersal (i.e. bacteria are more resistant to sympatric compared with allopatric phage populations) (Morgan *et al.*, 2005). Moderate increases in the relative rate of bacterial dispersal (1–10%) have no effect on local adaptation; this is because bacteria already have the upper hand in the coevolutionary arms race. By contrast, moderate increases in the relative rate of phage dispersal (1–10%) reverse patterns of local adaptation such that phages are locally adapted. This arises because dispersal introduces novel genetic variation [genetic variation for both resistance and infectivity has been shown to readily evolve in coevolving populations of *P. fluorescens* and SBW25Φ2 (Poullain *et al.*, 2008)] enhancing the adaptive potential of phage such that, on average, phages have the upper hand in the coevolutionary arms race (Morgan *et al.*, 2005). Mechanisms, such as dispersal, that enhance the adaptive potential of the lagging partner in a coevolutionary association can have a significant impact upon the dynamics of coevolution because such reciprocal evolutionary change may only proceed as rapidly as the slowest adapting partner. Such 'warming' of coevolutionary cold-spots (Gomulkiewicz *et al.*, 2000) through increased dispersal of the host (Brockhurst *et al.*, 2007a) or host and parasite simultaneously (Forde *et al.*, 2007; Morgan *et al.*, 2007) has been observed in several studies. However, the effect of greater relative rates of parasite dispersal remains unconsidered.

Although empirical evidence suggests that greater relative rates of parasite compared with host dispersal lead to greater parasite local adaptation (Dybdahl & Lively, 1996; Lively & Dybdahl, 2000; Morgan *et al.*, 2005), the impact on other aspects of the coevolutionary process such as the rate of coevolutionary change and the extent of coevolutionary escalation remain largely unexplored. The rate of coevolution has been shown to affect genetic diversity and population dynamics in coevolving populations (Buckling & Hodgson, 2007; Thompson, 2005), whereas the evolution of more broadly infective parasites has clear implications for disease (Woolhouse *et al.*, 2002). Moreover, when compared with the wide range of relative rates of parasite dispersal observed in natural systems, only a very restricted range of relative rates has thus far been studied using experimental metapopulations (Morgan *et al.*, 2005). To further investigate the effects of parasite dispersal on the coevolutionary process, we established replicate metapopulations of the common soil bacterium *P. fluorescens* SBW25 and its lytic viral bacteriophage SBW25Φ2, which were propagated by serial transfer. Within each metapopulation, phages were migrated from a migrant-pool at a range of different rates representative of those observed in natural systems, ranging from no dispersal in both host and parasite, to increasingly greater dispersal of parasites relative to hosts. Hosts were left unmigrated in all treatments. Previous studies with this host–parasite association have shown persistent cycles of coevolution imposing directional selection for increased infectivity and resistance ranges through time in phage and bacteria respectively (Buckling & Rainey, 2002), such ranges are a measure of the extent of coevolutionary escalation. We assayed levels of evolved bacterial resistance range and phage infectivity range (these are 'global' measures against both sympatric and allopatric antagonists), as well as the rate of coevolutionary change in one focal patch within each experimental metapopulation.

We hypothesized that phage dispersal would increase the adaptive potential of phages by introducing novel genetic variation, but that high levels of dispersal would impede adaptation by homogenizing genetic variation between patches and/or introducing locally maladaptive alleles. This leads to the prediction of a negative quadratic effect of phage gene-flow rate on the adaptive potential of phages. Because bacteria are ahead in the coevolutionary arms race in the absence of dispersal and phage adaptation is the rate-limiting-step of coevolution in this system, we further predicted: (1) a negative quadratic relationship between the rate of coevolution and phage dispersal rate; (2) a negative quadratic relationship of the extent of evolutionary escalation in resistance and infectivity ranges with phage dispersal rate.

Materials and methods

Culturing conditions

Cultures were grown in 30-mL glass universals with loose fitting plastic caps containing 6 mL of Kings B (KB) medium in a static incubator at 28 °C. Cultures were propagated by serial transfer, with 60 μL of culture being transferred to a fresh KB microcosm every 48 h. Samples

of each culture were frozen in 20% glycerol and stored at −80 °C every two transfers throughout the course of the experiment.

Isolation of bacteria and phage

Phage samples were isolated during the experiment by centrifuging samples of culture (13 000 rpm/9500 *g*, 2 min) in 10% chloroform. This lysed and pelleted the bacterial cells, leaving a suspension of phage particles in the supernatant. Isolated phage samples were then stored at 5 °C. Bacteria were isolated by growing cultures overnight in a KB microcosm containing 0.37% Virkon® (a commercially available disinfectant). At this concentration Virkon® is toxic towards bacteriophage particles whereas being nontoxic towards *P. fluorescens*. 60 μL was then transferred to a fresh KB microcosm and incubated for a further 24 h. This treatment left bacteria viable and free from phage and Virkon®. Presence of phage following this procedure was routinely checked by assaying the infectivity of a sample of culture against ancestral bacteria, no phages were detected.

Initiating populations

18 KB microcosms were inoculated with approximately 10^7 isogenic cells of *P. fluorescens* isolate SBW25 and 10^5 isogenic particles of the lytic DNA phage, SBW25Φ2. Cultures were initially propagated for eight transfers to allow divergence between populations prior to migration.

Experimental treatments

Following divergence, populations were assigned to one of six replicate metapopulations, each consisting of three microcosms. Each replicate metapopulation was then used to found five further metapopulations, each of which was subjected to one of five different phage migration regimes (0%, 0.1%, 1%, 10% and 100% of phage population from migrant-pool) for 24 days (12 transfers) of culturing. Bacteria were left unmigrated in all treatments: at each transfer samples of bacteria were isolated from each population and 60 μL of this isolate was transferred to a fresh microcosm. By contrast transferred phage came from two sources: unmigrated phage isolated from the relevant population, and phage from a migrant-pool for each metapopulation, which consisted of equal proportions by volume of phages isolated from each constituent microcosm. The proportion of the total transferred volume (60 μL) added from each source was determined by the migration treatment, for example under the 1% migration regime, 0.6 μL of transferred phage came from the migrant pool and 59.4 μL came from the phage isolated from the relevant population.

Assays

Quantifying resistance and infectivity

Bacterial resistance was assayed as a binary trait, such that a given bacterial colony could be either susceptible or nonsusceptible to infection by phage. For each assayed population, ten individual bacterial colonies were isolated by plating on a KB agar plate. Colonies were then streaked across a 20 μL line of phage on a KB agar plate and incubated for 24 h at 28 °C. A colony was defined as susceptible if there was visible inhibition of growth upon crossing the line of phage. Resistance was recorded as the proportion of nonsusceptible bacteria per population, whereas infectivity was measured as the proportion of susceptible bacteria per population. Within each migration treatment, one focal population from each of the six replicate metapopulations was selected to undergo assays.

Rate of coevolution

To determine if directional antagonistic coevolution occurred in this experiment, we used stored population samples (see above) to measure how the infectivity of phage populations to a bacterial population changed through time. Specifically, at transfers 2, 4, 6, 8 and 10 we determined the resistance (proportion resistant colonies) of bacterial populations to past (two transfers previous), contemporary and future (two transfers subsequent) sympatric phage populations. If directional antagonistic coevolution was occurring then we would expect, for multiple time points, future phage to be better than contemporary phage, and for contemporary phage to be better than past phage at infecting contemporary bacteria, hence a positive slope of infectivity against time (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, and averaged across time-points (Brockhurst *et al.*, 2003). Because bacterial resistance to contemporary phage remains relatively constant across time-points, we can infer bacterial adaptation (Brockhurst *et al.*, 2007b, 2003), hence when considered over multiple time-points this is a measure of coevolution, rather than simply phage infectivity evolution.

Resistance and infectivity ranges

The breadth of resistance and infectivity ranges was assayed every four transfers by determining the resistance/infectivity for each bacteria/phage population when assayed against all other focal populations from the other migration treatments that shared a founding metapopulation. This provides a 'global' measure of which treatment has produced the relatively most infectious and resistant populations, whereas controlling for the effect of founding metapopulation. Phage infectivity to their sympatric bacteria (i.e. the bacteria from the same microcosm and time point) was

measured every two transfers throughout the course of the experiment.

Statistical analysis

Sympatric infectivity, rate of coevolution and breadth of infectivity and resistance ranges were averaged through time and analysed separately using General Linear Models carried out in Minitab. Founding metapopulation was fitted as a random factor and Log10 (migration rate + 0.01) was simultaneously fitted as both a linear and quadratic covariate. Whether the addition of a quadratic term significantly improved model fit over a simpler linear model was determined using partial *F*-tests. Resistance ranges through time were log10 transformed and infectivity ranges through time were square-root transformed to meet the necessary assumptions (normality, homogeneity of variance).

Results

As predicted, we observed a negative quadratic relationship between the rate of phage dispersal and the rate of coevolution which peaked at 1% (Fig. 1; founding metapopulation, $F_{5,22} = 0.77$, $P = 0.579$; linear effect, $F_{1,22} = 0.37$, $P = 0.550$; negative quadratic effect, $F_{1,22} = 7.29$, $P = 0.013$, partial *F*-test for inclusion of quadratic rate term, $F_{1,22} = 7.29$, $P < 0.05$). Because coevolution is predominantly directional in this system (Buckling & Rainey, 2002), more rapid coevolution is typically associated with the evolution of broader phage infectivity range. In line with this, a negative quadratic relationship between the rate of phage dispersal and phage infectivity range was observed which also peaked at 1% (Fig. 2; founding metapopulation, $F_{5,22} = 3.26$, $P = 0.024$; linear term, $F_{1,22} = 26.18$, $P < 0.001$; negative quadratic term, $F_{1,22} = 38.51$, $P < 0.001$; partial *F*-test for inclusion of quadratic rate term, $F_{1,22} = 38.5$, $P < 0.01$).

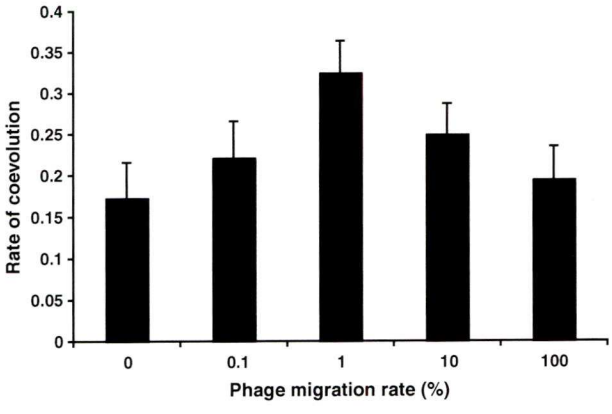

Fig. 1 The effect of phage migration rate on the rate of coevolution. The rate of coevolution was given by the slope of the change in infectivity of a phage population through time. Bars show mean (+SEM) rate of coevolution averaged through time.

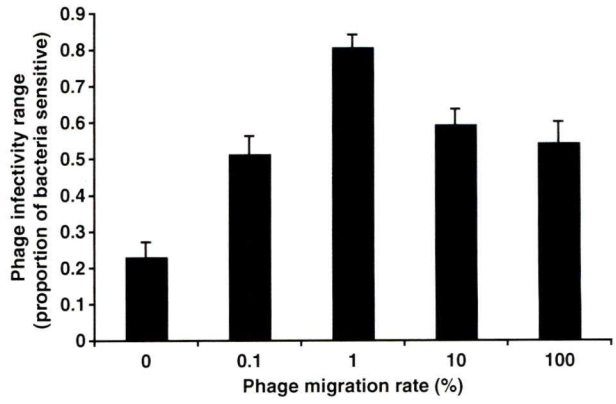

Fig. 2 The effect of phage migration rate on phage infectivity range. The infectivity range was given by determining the infectivity of each phage population when assayed against bacteria from all other focal populations from the other migration treatments that shared a founding metapopulation, providing a measure of 'global' infectivity. Bars show mean (+SEM) infectivity range of phage populations averaged through time.

These data are consistent with the hypothesis that phage dispersal between patches can increase genetic variation thereby enhancing phage evolutionary potential, but that high levels of dispersal (10–100%) may impede phage evolution, either through 'genetic swamping' or homogenization of genetic variation between patches, thereby limiting the rate and extent of coevolution attainable.

Because coevolution is a reciprocal process, bacterial resistance range was expected to evolve in response to changes in phage infectivity range. Bacterial resistance ranges also displayed a negative quadratic relationship with the rate of phage migration peaking at 1% (Fig. 3; founding metapopulation, $F_{5,22} = 2.96$, $P = 0.034$; linear term, $F_{1,22} = 8.94$, $P = 0.007$; negative quadratic term, $F_{1,22} = 9.91$, $P = 0.005$; partial *F*-test for inclusion of quadratic rate term, $F_{1,22} = 9.94$, $P < 0.01$) and were positively correlated with infectivity ranges (correlation of infectivity and resistance range means; Pearson's $r = 0.935$, $P = 0.02$). This suggests that bacterial resistance ranges were able to successfully evolve in response to the broadening of phage infectivity range through time despite a complete lack of dispersal. Taken together with previous studies (Brockhurst *et al.*, 2007a; Morgan *et al.*, 2007, 2005) this suggests that bacterial populations possess potential for coevolutionary escalation that remains unutilized in coevolving populations limited by the rate of phage adaptation.

The decline in the rate of coevolution and breadth of phage infectivity range at high rates of dispersal (10–100%) could have arisen through two mechanisms: 'genetic swamping' causing loss of locally beneficial alleles, or homogenization of genetic variation between patches. To assess whether locally beneficial infectivity alleles were lost at high phage gene-flow rates,

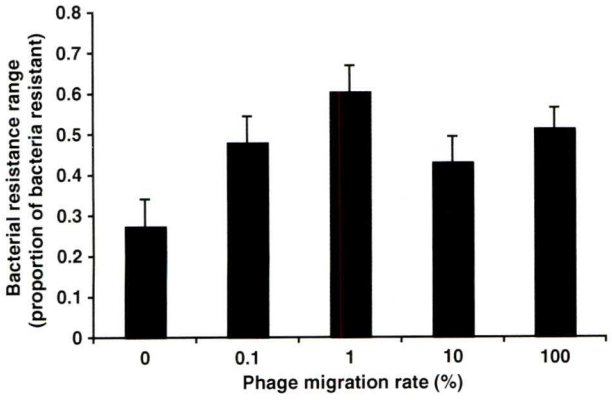

Fig. 3 The effect of phage migration rate on bacterial resistance range. The resistance range was given by determining the resistance of each bacteria population when assayed against phage from all other focal populations from the other migration treatments that shared a founding metapopulation, providing a measure of 'global' infectivity. Bars show mean (+SEM) resistance range of bacterial populations averaged through time.

infectivity of phages to their sympatric bacterial hosts was analysed; a decline in sympatric infectivity at high rates of dispersal would have been expected if locally beneficial infectivity alleles were being lost through 'genetic swamping'. Phage dispersal led to higher levels of infectivity of phages on their contemporary sympatric bacterial hosts (Fig. 4; founding metapopulation, $F_{5,22} = 3.31$, $P = 0.022$; linear effect, $F_{1,22} = 4.00$, $P = 0.058$; negative quadratic effect, $F_{1,22} = 7.97$, $P = 0.010$; partial F-test for inclusion of quadratic rate term, $F_{1,22} = 7.97$, $P < 0.01$). Further analysis, excluding the 0% dispersal data, found no difference in sympatric infectivity between other rates of dispersal (Fig. 4; founding metapopulation, $F_{1,16} = 3.29$, $P = 0.031$; linear effect, $F_{1,16} = 0.30$, $P = 0.593$; negative quadratic effect, $F_{1,16} = 0.00$, $P = 0.955$). Because no decline in sympatric infectivity was observed with increasing dispersal rate, this suggests that, in this experimental system, high rates of dispersal do not significantly limit phage adaptation to local bacterial hosts through 'genetic-swamping'. It seems likely therefore that the decline in the rate of coevolution and breadth of infectivity range observed at high rates of phage dispersal were due to homogenization of genetic variation between patches.

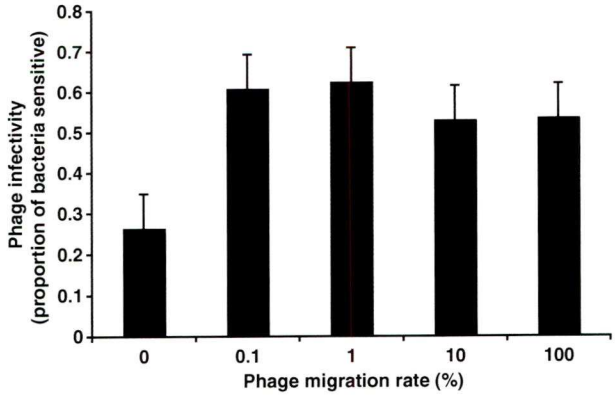

Fig. 4 The effect of phage migration rate on sympatric infectivity. The sympatric infectivity was given by determining the infectivity of a phage population on bacteria from the same time-point and microcosm. Bars show mean (+SEM) infectivity of phage populations to contemporary sympatric bacterial populations averaged through time.

Discussion

Evidence from theory (Gandon & Michalakis, 2002), natural populations (Dybdahl & Lively, 1996; Lively & Dybdahl, 2000) and laboratory populations (Morgan *et al.*, 2005) suggests that greater relative rates of dispersal in parasites compared with hosts should increase parasite local adaptation (Greischar & Koskella, 2007; Hoeksema & Forde, 2008). However, local adaptation provides only a contemporary 'snap-shot' of coevolutionary interactions, yielding little information about other aspects of the coevolutionary process. The results presented here extend local adaptation findings to consider the effect of a wide range of rates of parasite dispersal on the dynamics and outcomes of coevolution. We demonstrate that parasite dispersal can enhance the evolutionary potential of parasites increasing both the rate and extent of escalation attainable during antagonistic host–parasite coevolution. However, high rates of parasite dispersal can impede parasite adaptation, our results suggest that the most likely mechanism for this is through homogenizing genetic variation between patches, thereby constraining the coevolutionary process. In a previous study where bacteria and phage were migrated simultaneously (Morgan *et al.*, 2007), evolved phage infectivity range did not decline at high rates of dispersal (10–50%) as observed here. This suggests that the decoupling of host and parasite dispersal can alter the outcome of coevolution by limiting the effects of dispersal on evolutionary potential to one or other antagonist.

Our results suggest that bacterial populations possess coevolutionary potential that remains unutilized in the absence of phage dispersal, posing the question: why if broader resistance ranges can be evolved do they not evolve in the absence of phage dispersal (as seen by the low evolved resistance ranges for 0% migration rate in Fig. 3)? The strong positive correlation between resistance range and infectivity range in this experiment suggests that selection favours the evolution of sufficient rather than maximal resistance ranges. This is likely to be due to the high cost of phage resistance mutations in this system (Brockhurst *et al.*, 2004; Buckling *et al.*, 2006), such that at any given time bacterial clones with broader

than necessary resistance mutations are likely to be outcompeted by sufficiently resistant but fitter clones.

Acceleration of coevolution due to parasite dispersal is likely to be particularly apparent in coevolutionary systems where parasites are the lagging antagonist in the absence of dispersal. This is due to the rate of coevolutionary change being limited by the adaptive rate of the slowest partner. Under such conditions dispersal is likely to lead to the more rapid evolution of more infective parasites. The generality of the patterns of infectivity and resistance range evolution observed in this study may be somewhat limited to systems that undergo predominantly directional selection. Such systems include certain plant–pathogen interactions (see for example, Thrall & Burdon, 2003; Laine, 2006) and other host–parasite interactions that broadly comply with a multilocus gene-for-gene model of coevolutionary interaction, which allows for the evolution of generalist resistance and infectivity phenotypes in hosts and parasites respectively (Damgaard, 1999; Sasaki, 2000; Thompson & Burdon, 1992).

In this and previous studies with this host–parasite association, adaptation has consistently peaked at 1% dispersal despite differences in the precise ecological conditions used in each study (Brockhurst *et al.*, 2007a; Morgan *et al.*, 2007). However, it is unclear how, low, intermediate or high rates of dispersal should be defined for natural systems. Undoubtedly this is likely to be under the influence of a wide range of contributory factors that also affect genetic diversity (e.g. mutation rate, population size generation time, etc.). Given this proviso, these results could have implications for health and agriculture. Moderate increases in parasite dispersal associated with increased mobility of human populations and movement of livestock and crops could significantly alter coevolutionary dynamics leading to the more rapid emergence of more infective parasites. Both theoretical and empirical evidence suggests that through increasing transmission opportunities this is likely to be associated with an increase in the virulence of disease (Boots et al., 2004; Boots & Mealor, 2007; Boots & Sasaki, 1999; Herre, 1993). By contrast, very large increases in parasite dispersal rate are likely to erode the potential benefits to parasites of dispersal, leading to decline of parasite evolutionary potential, thereby limiting infectivity and virulence evolution.

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Appendix 3: Vogwill et al (2009)

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E-NOTE

Source Populations Act as Coevolutionary Pacemakers in Experimental Selection Mosaics Containing Hotspots and Coldspots

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ABSTRACT: Natural populations of hosts and their enemies are often spatially structured, with patches that vary in the strength of reciprocal selection, so-called coevolutionary hotspots and coldspots with strong or weak reciprocal selection, respectively. Theory predicts that dispersal from hotspots should intensify coevolution in coldspots, whereas dispersal from coldspots should weaken coevolution in hotspots; however, there have been few empirical tests. We addressed this using paired populations of the bacterium *Pseudomonas fluorescens* and the phage SBW25Φ2 linked by one-way dispersal. Within each population, the strength of reciprocal selection was manipulated by altering the bacteria-phage encounter rate, which changes the rate of coevolution without affecting environmental productivity. We observed that dispersal from hotspots accelerated coevolution in coldspots, while dispersal from coldspots decelerated coevolution in hotspots. These results confirm theoretical predictions and suggest that source populations can act as coevolutionary "pacemakers" for recipient populations, overriding local conditions.

Keywords: geographic mosaic theory, host-parasite, coevolution, resistance, infectivity, experimental evolution.

Introduction

Antagonistic coevolution, the process of reciprocal selection for defense and counterdefense between hosts and their enemies, is pervasive in biological communities and thought to have a wide range of ecological and evolutionary consequences, including driving population dynamics (Thompson 1998; Loeuille et al. 2002; Yoshida et al. 2003, 2007), the evolution of diversity (Frank 1993; Benkman 1999; Schluter 2000; Buckling and Hodgson 2007), and the evolution of parasite virulence (Bull 1994; Gandon and Michalakis 2000; Gandon et al. 2002; Woolhouse et al. 2002). Coevolving populations of hosts and their enemies are often spatially structured, occurring as a set of patches connected by dispersal. The geographic mosaic theory posits that variation in ecological conditions between patches can lead to differences in local selection, generating mosaics in adaptation (Thompson 2005). This can potentially lead to variation in the strength of reciprocal selection between hosts and parasites in different patches, such that some patches display reciprocal selection (hotspots), while others do not (coldspots; Gomulkiewicz et al. 2000). Dispersal and gene flow between these patches can then act to redistribute genotypes and alleles across the selection mosaic (Thompson 1999, 2005).

A key theoretical prediction is that coevolutionary hotspots need not be ubiquitous to have an effect on the evolutionary dynamics of an interaction across the selection mosaic as a whole (Thompson 2005). Specifically, coevolutionary hotspots can drive coevolution in coldspots, provided there is gene flow and sufficiently strong selection within the hotspot (Gomulkiewicz et al. 2000). However, coldspots can also influence evolutionary dynamics in hotspots under certain conditions. For example, when hotspots are surrounded by coldspots, gene flow can lead to the swamping of the hotspot with coldspot-adapted genotypes, which can override local conditions by weakening the response to reciprocal selection pressures (Nuismer et al. 2003). Taken together, these findings lead to the theoretical prediction that dispersal from hotspot to coldspot should intensify coevolution in the coldspot, whereas dispersal from coldspot to hotspot should weaken coevolution in the hotspot.

Geographic variation in the strength of reciprocal selection has been inferred in a number of natural host-enemy systems (Benkman 1999; Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thompson and Cunningham 2002; Thrall and Burdon 2003; Thompson 2005; Laine

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2006; Toju and Sota 2006; Hanifin et al. 2008). Indeed, much empirical data suggest that classification into coevolving hotspots and noncoevolving coldspots may be rather too simplistic (Nash 2008) and that there is often likely to be a continuum of intensity of reciprocal selection strength between pure coldspots and extreme hotspots (Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thrall and Burdon 2003; Toju 2008). A number of ecological factors have been suggested to cause variation in reciprocal selection pressures; these include abiotic factors, such as environmental productivity (Hochberg and van Baalen 1998; Lopez-Pascua and Buckling 2008) and climate (Toju and Sota 2006; Toju 2008), and biotic factors, such as host-enemy encounter rates (Laine 2006) and the presence/absence of other interacting species (Benkman et al. 2001; Thrall et al. 2007). However, while geographic variation in reciprocal selection appears to be widespread in natural populations and its importance is highlighted by theory (Thompson 2005), there have been few explicit empirical tests of its impact on coevolutionary dynamics in selection mosaics connected by dispersal.

One reason for this lack of direct empirical data is that controlled, replicated coevolution experiments are extremely difficult to conduct in natural populations where the spatial and temporal scales are large and rates of dispersal and historical relationships between patches are difficult to determine and control. For these reasons, laboratory populations of bacteria and their viral parasites, phage, have emerged as key model systems for testing aspects of the geographic mosaic theory (Forde et al. 2004, 2007; Morgan et al. 2005, 2007; Brockhurst et al. 2007*b*; Lopez-Pascua and Buckling 2008; Vogwill et al. 2008). The bacterium *Pseudomonas fluorescens* SBW25 and its naturally associated phage SBW25Φ2 have been used extensively to test coevolutionary theory (Brockhurst et al. 2007*a*). Persistent arms race coevolution with directional selection for increased bacterial resistance and phage infectivity range has been observed, suggesting a multilocus gene-for-gene interaction (Buckling and Rainey 2002; Poullain et al. 2008). Crucially, because population samples can be cryogenically stored in "suspended animation," it is possible to directly measure rates of coevolutionary change through time. Increasing within-population mixing by periodically shaking culture vessels has been shown to increase the strength of reciprocal selection by raising the bacteria-phage encounter rate; this strengthens selection for resistance and, by extension, for novel infectivity—thereby accelerating coevolution, approximately doubling its rate—but has no affect on environmental productivity (Brockhurst et al. 2003). Here, we use this simple environmental manipulation to create patches within experimental landscapes that vary in the strength of reciprocal selection (strong reciprocal selection/with population mixing, henceforth PM^+; weak reciprocal selection/without population mixing, henceforth PM^-).

Experimental landscapes each consisted of two populations of *P. fluorescens* and SBW25Φ2 connected by unidirectional dispersal such that one population acted as a source of migrants and the other as a recipient of migrants. Four possible source-recipient arrangements were investigated: (1) PM^- source–PM^- recipient, (2) PM^+ source–PM^+ recipient, (3) PM^- source–PM^+ recipient, and (4) PM^+ source–PM^- recipient. Arrangements 1 and 2 represent homogeneous landscapes, while 3 and 4 are heterogeneous with regard to population mixing and therefore the strength of reciprocal selection. In addition, two rates of between-population dispersal were investigated. Populations were propagated by batch culture for a total of 12 transfers, and every two transfers the rate of coevolution in each recipient population was measured. We also measured the baseline rate of coevolution in isolated PM^+ and PM^- populations that received no migrants.

Material and Methods

Culturing Techniques

Populations were propagated by batch culture in 30-mL glass universal bottles with loose-fitting plastic caps containing 6 mL of standard King's B medium (KB) in an incubator at 28°C. PM^- populations were incubated statically; PM^+ populations were shaken for 1 min every 30 min at 200 rpm (Brockhurst et al. 2003). A 60-μL aliquot of each population was transferred to fresh media every 48 h. Samples of culture were stored at −80°C in 20% glycerol. Phage populations were isolated by centrifuging samples of culture in 10% chloroform (which lysed and pelleted bacterial debris) and then stored at 4°C.

Experimental Design

Forty-eight replicate populations (24 PM^- populations and 24 PM^+ populations) were founded with 10^5 clonal particles of phage and 10^7 *Pseudomonas fluorescens* SBW25 cells and allowed to coevolve for six transfers before beginning dispersal treatments. After this period, populations were assigned into source-recipient pairs to create six replicates of each of the following source-recipient arrangements: (1) PM^- source–PM^- recipient, (2) PM^+ source–PM^+ recipient, (3) PM^- source–PM^+ recipient, and (4) PM^+ source–PM^- recipient. Each source-recipient pair was used to found two experimental landscapes, one to undergo 1% dispersal and one to undergo 10% dispersal. Six PM^+ and six PM^- recipient populations were also used to found isolated populations that received no migrants. We transferred 60-μL aliquots to fresh microcosms every

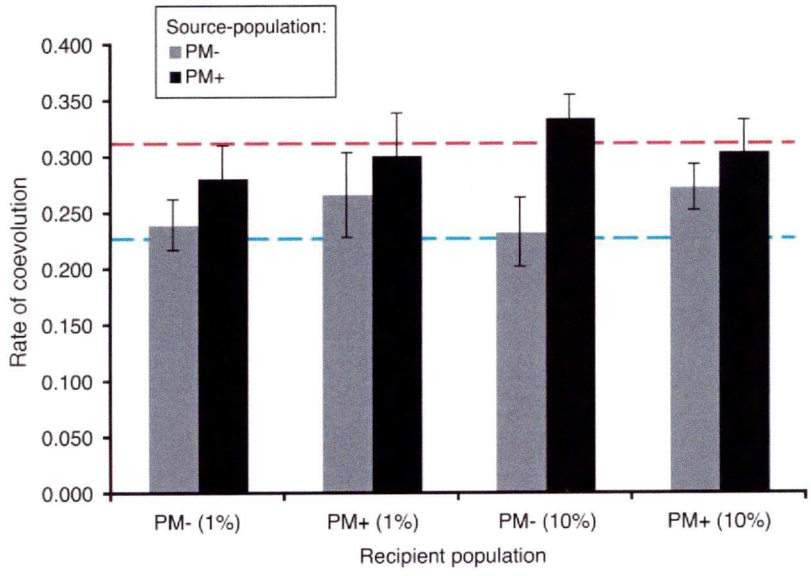

Figure 1: Rate of coevolution in recipient populations. Bars represent the mean rate of coevolution averaged through time ± SE in recipient populations. Dashed lines represent the mean rate of coevolution in isolated PM^+ (*red*) and PM^- (*blue*) populations that received no migrants. Source population refers to the population-mixing regime in the source population, while recipient population refers to the population-mixing regime in the recipient population. Rates of between-patch dispersal are provided in parentheses.

48 h for a total of 12 transfers. Source to recipient population dispersal was achieved by, for each recipient population, a defined portion of this transferred aliquot being contributed by the corresponding source population. Depending on the dispersal rate, this involved transferring either 54 μL of recipient population and 6 μL of source population to a fresh microcosm (10% dispersal rate) or 59.4 μL of recipient population and 0.6 μL of source population (1% dispersal rate).

Measuring Coevolution

Bacterial resistance for a given population was determined by isolating 10 bacterial colonies on KB agar, which were then streaked across a perpendicular line of phage that had been previously dried onto a KB agar plate. Any bacterial colonies that showed growth inhibition on encountering the line of phage were classed as sensitive. Resistance was measured as the proportion of resistant bacterial colonies. Antagonistic coevolution between *P. fluorescens* and SBW25Φ2 has been shown to be predominantly escalatory with directional selection for increasing infectivity and resistance through time (Buckling and Rainey 2002; Brockhurst et al. 2003). To determine the rate of coevolution, we measured how the infectivity of phage populations to a bacterial population changed through time. Specifically, every two transfers, we determined the resistance of bacterial populations to past (two transfers previous) and future (two transfers subsequent) phage populations from the same replicate line. If directional coevolution was occurring, then we would expect, for multiple time points, future phage to be better than past phage at infecting contemporary bacteria, hence a positive slope of infectivity against time: the magnitude of this slope gives a measure of the rate of coevolutionary change (Brockhurst et al. 2003, 2007*b*; Lopez-Pascua and Buckling 2008; Vogwill et al. 2008).

Statistical Analysis

Rates of coevolution were averaged through time and analyzed using a linear mixed model performed in SPSS. Source population mixing, recipient population mixing, and dispersal rate were fitted as fixed factors, while founding population was fitted as a random factor nested within both source and recipient population mixing.

Results

In the absence of immigration, population mixing had a significant effect on the strength of reciprocal selection within populations ($F_{1,10} = 12.62$, $P < .01$), confirming that the PM^+ treatment created hotspots (mean rate of coevolution = 0.312 ± 0.016) while the PM^- treatment created coldspots (mean rate of coevolution = 0.226 ± 0.018). Within experimental landscapes, the coevolution-

Table 1: Test of fixed effects

Source	Numerator df	Denominator df	F	P
Intercept	1	20	510.825	<.001
SPM	1	20	4.503	.047
RPM	1	20	.328	.573
DR	1	20	.840	.370
SPM × RPM	1	20	.600	.448
SPM × DR	1	20	.840	.370
RPM × DR	1	20	.352	.560
SPM × RPM × DR	1	20	1.049	.318

Note: SPM = source population mixing; RPM = recipient population mixing; DR = dispersal rate.

ary rate of recipient populations was determined by population mixing in the source population (fig. 1; $F_{1,20} = 4.503$, $P = .047$) but not by population mixing in the recipient population itself (fig. 1; $F_{1,20} = 0.328$, $P = .573$) or by the rate of immigration (fig. 1; $F_{1,20} = 0.840$, $P = .370$), and there were no significant interactions between main effects (table 1). Therefore, as predicted, immigration from PM^+ source populations increased the rate of coevolution in PM^- recipient populations, while immigration from PM^- source populations decreased the rate of coevolution in PM^+ recipient populations, relative to equivalent recipient populations in homogeneous landscapes.

Discussion

Central to the geographic mosaic theory is the concept of selection mosaics with patches that vary in intensity of reciprocal selection, so-called coevolutionary hotspots and coldspots (Thompson 2005). Such geographic variation in reciprocal selection intensity appears to be widespread in natural host-enemy populations (Benkman 1999; Kraaijeveld and Godfray 1999; Brodie et al. 2002; Thompson and Cunningham 2002; Thrall and Burdon 2003; Laine 2006; Toju and Sota 2006). In this study, we experimentally manipulated the strength of reciprocal selection within populations through altering host-parasite encounter rates without affecting environmental productivity. Our results suggest that heterogeneity in the strength of reciprocal selection across a landscape is an important determinant of coevolutionary dynamics within population patches. Specifically, for recipient populations in heterogeneous landscapes, immigration from a patch with stronger reciprocal selection can accelerate coevolution, while immigration from a patch with weaker reciprocal selection can decelerate coevolution. This suggests that source populations can act as coevolutionary "pacemakers" for recipient populations, overriding local conditions.

It is notable that only low to moderate rates of dispersal were required to override local selection: as little as 1% immigration every ~7.5 host generations. It is somewhat surprising, however, that there was no significant effect of different rates of dispersal on coevolution in our experiment. Specifically, theory predicts that coevolutionary dynamics in coldspots should be more likely to resemble those in hotspots as the migration rate increases from low to moderate levels (Gomulkiewicz et al. 2000). It is possible that the two rates of dispersal (1% and 10%) used in our experiment were too similar to detect a significant difference, both being in effect moderate rates of dispersal, and that an even lower dispersal rate would be required to detect the pattern predicted by theory. It is interesting to note that another recent study on the effects of dispersal rate on adaptation also found little difference between the effects of 1% and 10% dispersal (Venail et al. 2008).

Hosts and parasites were codispersed at equal rates in our experiment. While in some host-parasite associations such congruent patterns of host and parasite gene flow are observed (Mulvey et al. 1991), in certain others, patterns of host and parasite gene flow are decoupled, with either the host (Delmotte et al. 1999) or the parasite (Dybdahl and Lively 1996; Davies et al. 1999) displaying relatively greater levels of gene flow. As in previous studies (Forde et al. 2004, 2007; Morgan et al. 2007), our findings may therefore be somewhat limited to host-parasite systems that experience simultaneous host-parasite dispersal. Such situations are likely to arise where the parasite is reliant on the host for its dispersal, as is the case for contact-transmitted parasites, or where codispersal of host and parasite is driven by an external factor such as a prevailing wind or an aquatic current.

These results confirm, along with the findings of a previous experimental study that manipulated environmental productivity (Forde et al. 2007), that dispersal from hotspots can "warm up" coevolution in coldspots. However, ours is the first, as far as we are aware, to show empirically that dispersal from populations with weaker reciprocal selection can "cool down" those with more intense reciprocal

selection. This has been shown to be theoretically possible (Hochberg and van Baalen 1998; Gomulkiewicz et al. 2000; Nuismer et al. 2003; Thompson 2005); however, it is important to consider whether hotspots or coldspots are likely to predominate in natural selection mosaics. In selection mosaics generated by productivity gradients, where there is likely to be a positive relationship between productivity and population density (Lopez-Pascua and Buckling 2008), it is probable that hotspots will have a greater impact because they will act as net sources of migrants, while coldspots will act as net recipients. However, where reciprocal selection is weakened through reduced host-parasite encounter rate (Laine 2006), such coldspot populations may act as net sources of migrants as a result of lower incidence of parasitism, which can negatively regulate host population growth in nature (Tompkins et al. 2002). In addition, geographical limitations to dispersal may often result in unidirectional movement of migrants (e.g., aquatic currents, prevailing winds); under such conditions, coldspots or hotspots that act as net sources of migrants are likely to determine coevolutionary dynamics across the selection mosaic.

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