

THE IMPACT OF MIGRATION FROM PARASITE-FREE PATCHES ON ANTAGONISTIC HOST-PARASITE COEVOLUTION

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Natural populations of hosts and parasites are often subdivided and patchily distributed such that some regions of a host species' range will be free from a given parasite. Host migration from parasite-free to parasite-containing patches is expected to alter coevolutionary dynamics by changing the evolutionary potential of antagonists. Specifically, host immigration can favor parasites by increasing transmission opportunities, or hosts by introducing genetic variation. We tested these predictions in coevolving populations of *Pseudomonas fluorescens* and phage $\Phi 2$ that received immigrants from phage-free populations. We observed a negative quadratic relationship between sympatric resistance to phage and host immigration rate (highest at intermediate immigration) but a positive quadratic relationship between coevolution rate and host immigration rate (lowest at intermediate immigration). These results indicate that for a wide range of rates, host immigration from parasite-free patches can increase the evolutionary potential of parasites, and increase the coevolutionary rate if parasite adaptation is limiting in the absence of immigration.

KEY WORDS: Arms race, experimental evolution, gene flow, geographic mosaic, local adaptation, microbes.

Antagonistic host-parasite coevolution, the reciprocal evolution of host defense and parasite counterdefense, is pervasive in ecological communities. Identifying the conditions that alter the relative advantage of hosts and parasites in a coevolutionary arms race is central to our understanding of disease (Woolhouse et al. 2002). Often, whichever partner adapts faster will have the upper hand

in the arms race (but see Lively 1999 for related discussion); several factors have been identified that contribute to determining relative evolutionary potential of hosts and parasites (Gandon and Michalakis 2002). Among them, metapopulation dynamics are thought to play an important role in the evolution and ecology of antagonistic host-parasite interactions (Gandon 2002; Thompson 2005), and migration is likely to be a key factor (Gandon and Michalakis 2002). Migration can provide genetic variation to potentially increase the rate of adaptive evolution (Lenormand 2002).

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As such, increases in the rate of migration of one coevolutionary partner relative to the other can confer an evolutionary advantage and lead to local adaptation (Gandon 2002; Gandon and Michalakis 2002; Morgan et al. 2005).

Many theoretical studies have considered metapopulations in which patches contain both host and parasite. However, species are often patchily distributed in space, which is likely to lead to some regions being free of a given parasite (henceforth termed parasite-free) (Nuismer et al. 2003b). Patches containing both hosts and parasites can be considered as coevolutionary hot spots and parasite-free patches as coevolutionary cold spots (Nuismer et al. 2003b). Migration between hot spots and cold spots is expected to alter coevolutionary dynamics (Gomulkiewicz et al. 2000; Nuismer et al. 2003b). Several empirical studies have demonstrated the importance of such spatial dynamics for mediating coexistence of otherwise unstable antagonists in metapopulations (Holyoak and Lawler 1996; Bonsall et al. 2002), but the impact of parasite-free patches on coevolutionary dynamics remains untested. Here, we consider how the rate of unidirectional host migration from parasite-free patches into patches containing both host and parasite affects the level of resistance and the rate of coevolution. Although simple, this pattern of host movement is biologically realistic and broadly relevant to a range of phenomena including biological invasions (Telfer et al. 2005) and the evolution of species' range (Case et al. 2005). Crucially, it combines two factors thought to be important in determining coevolutionary dynamics: (1) patchy parasite distribution, and (2) unequal relative rates of migration.

Immigration of hosts from parasite-free patches could alter the coevolutionary balance of the host-parasite interaction in two opposing ways. First, at least in the short term, the immigration of hosts can benefit (i.e., by conferring an evolutionary advantage) parasites by increasing transmission opportunities. Increasing transmission opportunities means more parasite progeny and hence more adaptive potential through an increased likelihood of rare beneficial mutations (Rhodes and Anderson 1996; Boots and Sasaki 1999; Thrall and Burdon 2002; Brockhurst et al. 2003; Brockhurst et al. 2006). The benefit to parasites is therefore likely to be a positive function of the immigration rate. Second, host immigration can benefit the host population by introducing novel genetic variation, which will increase the rate of host adaptation (Lenormand 2002). However, the benefit of host immigration will display diminishing returns and host adaptation may be reduced at high rates of immigration through the effects of competition and the loss of beneficial endemic genotypes as susceptible immigrants swamp the population (Alleaume-Benharira et al. 2006). It is expected therefore that immigration of hosts from parasite-free patches will benefit parasites most at high rates of immigration, but may benefit either the host or parasite at lower rates of immigration.

The rate of host immigration from parasite-free patches is likely to impact the rate of coevolution; the effect of migration will depend on which partner is ahead in the arms race and therefore possesses the greater evolutionary potential in the absence of immigration (Gandon and Michalakis 2002; Morgan et al. 2005). Specifically, increasing the evolutionary potential of the leading partner is likely to have no effect or reduce the rate of coevolution, while increasing the evolutionary potential of the trailing partner may increase the rate of coevolution. This is because the rate of trailing partner adaptation is the rate-limiting step of coevolution.

Testing the predictions of coevolutionary theory in the field would be extremely difficult due to the spatial and temporal scales involved, combined with the difficulties of measuring real-time coevolution and accounting for uncontrolled variation (but see Thompson and Cunningham 2002). Populations of bacteria and their viral parasites (phage) can be used to overcome these problems (Bohannan and Lenski 2000; Buckling and Rainey 2002a). Recent studies have highlighted the importance of migration in coevolving bacteria-phage systems (Brockhurst et al. 2003; Forde et al. 2004; Morgan et al. 2005; Morgan et al., in press). We studied how the rate of host immigration from parasite-free patches affects the evolution of sympatric resistance and coevolutionary dynamics in replicate populations of the bacterium *Pseudomonas fluorescens* and the lytic phage SBW25Φ2. Persistent antagonistic coevolution has been observed in a number of studies using this system (Buckling and Rainey 2002a; Brockhurst et al. 2003; Buckling et al. 2006), and, in the absence of migration, bacteria are ahead in the arms race and phage are locally maladapted (Morgan et al. 2005). We hypothesized that immigration of bacteria from phage-free populations would (1) benefit phage as a positive function of immigration rate due to increased transmission opportunities, and (2) accelerate coevolution when the benefit to phage outweighed the benefit to bacteria.

Materials and Methods

ESTABLISHMENT OF FOUNDING POPULATIONS

Twelve replicate microcosms (30-mL glass universals with 6 mL of King's B nutrient broth) were inoculated with 10^7 isogenic cells of *Pseudomonas fluorescens* SBW25; six were simultaneously inoculated with 10^5 isogenic particles of the lytic phage SBW25Φ2. These were incubated at 28°C and shaken at 200 rpm for 1 min in every 30 min (Brockhurst et al. 2003). The static portion of the incubation period was required for appreciable phage adsorption to occur (Buckling and Rainey 2002a), whereas the shaken portion increased the rate of phage transmission thus increasing the strength of coevolutionary selection (Brockhurst et al. 2003). An aliquot (60 μL) of each population was transferred to a fresh microcosm every 48 h for eight transfers.

MIGRATION SELECTION EXPERIMENT

Each phage-containing founding population (1–6) was used to establish six sink populations (i.e., containing both bacteria and phage); these received immigrants from the corresponding phage-free source population (i.e., containing bacteria only) (1–6) at one of the following rates by volume of the transferred inoculum at each transfer: 0%, 0.01%, 0.1%, 1%, 10%, 50%. For example, at the 50% migration rate the fresh microcosm received 30 μ L of inoculum from the phage-containing sink population and 30 μ L from the phage-free source population. Phage populations were isolated by centrifuging with 10% chloroform (to lyse and pellet bacterial debris) and added in appropriate volumes at each transfer to make up the phage population shortfall caused by immigration from phage-free populations. Source and sink populations were incubated as above for a further eight transfers. Populations were stored at -80°C in 20% glycerol; phage populations were stored at 4°C .

MEASUREMENT OF RESISTANCE

Resistance of bacteria (infectivity of the phage population) was determined by streaking 10 independent bacterial colonies across a line of the sympatric phage population that had previously been inoculated onto a KB agar plate. A colony was defined as sensitive if there was inhibition of bacterial growth; otherwise it was defined as resistant (Buckling and Rainey 2002a).

MEASUREMENT OF COEVOLUTION RATE

To determine if antagonistic coevolution occurred, we measured how the infectivity of phage populations to a bacterial population changed through time. Specifically, at transfers 2, 4, and 6 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 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 (Brockhurst et al. 2003), we can infer bacterial adaptation; hence when considered over multiple time points this is a measure of coevolution, rather than simply phage infectivity evolution. To ensure that this was the case in this experiment we calculated the slope of contemporary resistance against time point for each population and found that these were not significantly different

from zero indicating that contemporary resistance does not change greatly between time points ($t = 1.24$, $df = 31$ $P = 0.22$).

STATISTICAL ANALYSES

To establish the change in sympatric resistance and coevolution rate caused by immigration, measures of resistance and coevolution rate for each migrated population were divided by the relevant measure of resistance and coevolution rate for the 0% migration control of the same founding population. Past, contemporary, and future relative resistance and coevolution rate averaged across time were analyzed in separate general linear models carried out in JMP (SAS Institute, Cary, NC), fitting $\log_{10}(\text{migration treatment} + 0.01)$ as both linear and quadratic covariates and founding population as a factor. Note that the significance of the linear term was determined prior to fitting the quadratic term.

Results and Discussion

The immigration of hosts from parasite-free patches had a complex effect on levels of sympatric resistance and rates of coevolution (Fig. 1). To better understand this effect relative levels of sympatric resistance and rates of coevolution were calculated (Figs. 2, 3). Relative sympatric bacterial resistance was maximal at intermediate rates of host immigration, resulting in a unimodal relationship, peaking at 1%, between immigration rate and relative sympatric resistance to past (Fig. 2; linear term $F_{1,26} = 1.89$, $P > 0.1$; negative quadratic term $F_{1,26} = 6.32$, $P = 0.01$), contemporary (Fig. 2; linear term $F_{1,26} = 0.14$, $P > 0.7$; negative

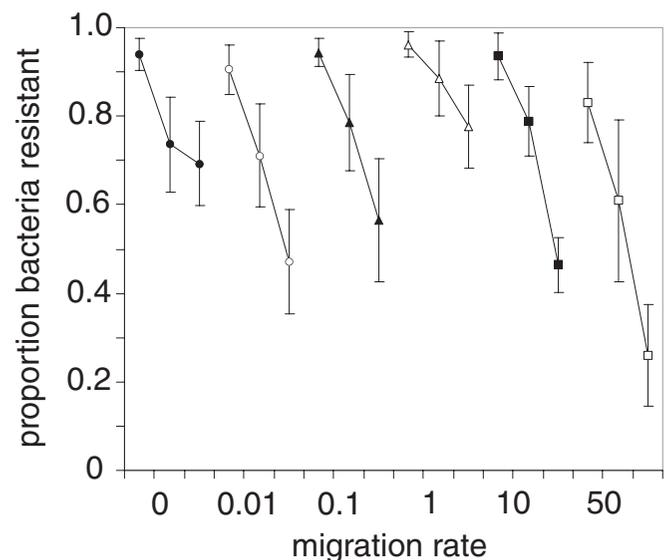


Figure 1. Coevolutionary interactions over a range of host immigration rates. Each set of lines shows (from left to right) proportion of bacteria resistant to phage populations from two transfers in the past, contemporary phage, and phage from two transfers in the future, averaged through time. Error bars represent \pm SEM.

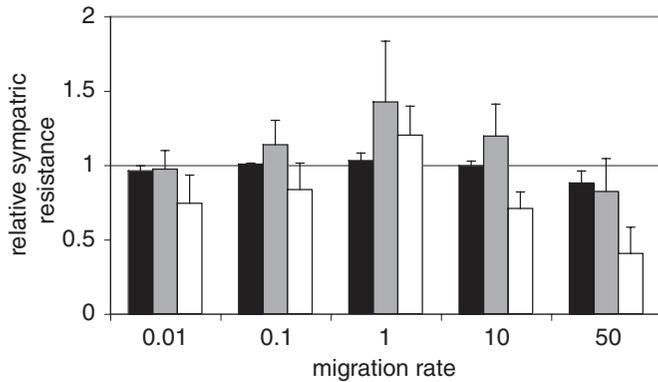


Figure 2. The effect of host immigration rate on sympatric resistance. Bars represent mean (\pm SEM) relative resistance to past (dark grey), contemporary (light grey), and future (white) sympatric phage populations. A value of 1 represents equality with 0% immigration control populations.

quadratic term $F_{1,26} = 4.67$, $P = 0.04$), and future (Fig. 2; linear term $F_{1,26} = 2.58$, $P > 0.1$; negative quadratic term $F_{1,26} = 8.37$, $P = 0.007$) phage populations. As expected, high (10–50%) rates of immigration favored phage more than bacteria by increasing the proportion of the host population susceptible to infection. Predicting the rest of the relationship a priori was more difficult as low and intermediate rates of immigration could have favored either bacteria or phage. Here, low rates (0.01–0.1%) of immigration favored phage relatively more than bacteria, probably through increasing the proportion of the host population susceptible to infection, without conferring any significant benefit to bacteria because phage killed beneficial genotypes prior to the acquisition of a resistance mutation. Intermediate rates (1%) of immigration favored bacteria more than phage; this was likely due to an increase in genetic variation that accelerated bacterial adaptation relative

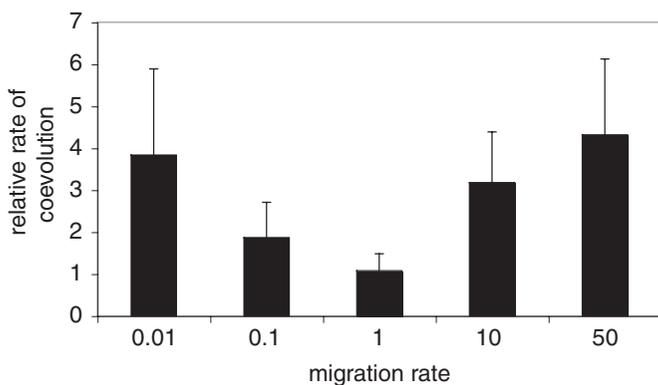


Figure 3. The effect of host immigration on the rate of coevolution. Bars represent mean (\pm SEM) relative rate of coevolution. A value of 1 represents equality with 0% immigration control populations.

to phage counteradaptation, leading to higher levels of sympatric resistance.

The net evolutionary advantage to phage at low (0.01–0.1%) and high (10–50%) rates of immigration was particularly evident in terms of reduced relative resistance to future sympatric phage populations. In the absence of immigration, phage have been shown to lag behind bacteria in this coevolutionary arms race (Buckling and Rainey 2002a; Morgan et al. 2005). Phage adaptation rate therefore imposes a limit on the rate of coevolution, hence acceleration of the coevolutionary rate was only expected when phage gained an evolutionary advantage through host immigration. In support of this, coevolution was accelerated at low (0.01–0.1%) and high (10–50%) immigration rates, resulting in an inverted unimodal relationship between immigration rate and relative rate of coevolution (Fig. 3; linear term $F_{1,26} = 0.42$, $P > 0.5$; positive quadratic term $F_{1,26} = 4.33$, $P = 0.04$). Crucially, when the benefit of immigration accrued mostly to bacteria (1%) no change in the rate of coevolution was observed relative to populations without immigration (paired t -test of coevolution rate averaged through time; $t = 0.8$, $df = 5$, $P > 0.4$) supporting the prediction that changes in coevolutionary rate depend on advantages to the lagging species.

The geographical mosaic theory (Thompson 2005) highlights the importance to coevolutionary dynamics of the flow of individuals and genes of both hosts and parasites in spatially homogeneous (Nuismer and Thompson 1999; Gomulkiewicz et al. 2000) and heterogeneous (Hochberg and van Baalen 1998; Nuismer et al. 2003a) environments. These studies explore how different host and parasite migration scenarios affect the coevolutionary process. Our study investigates the less well-explored possibility that at least in certain places and/or at certain times, the geographic mosaic may be influenced by the migration of susceptible hosts alone (Nuismer et al. 2003b). Indeed, parasite-free patches could arise in spatially structured systems in which the parasite cannot persist with the host (Hochberg and van Baalen 1998), or in which local extinctions of both species are frequent and there is a considerable lag time between recolonization by the host followed by the parasite (Hochberg and Moller 2001). The results presented here suggest that immigration from a parasite-free cold spot to a hot spot can actually intensify antagonistic interactions and accelerate coevolution by increasing the evolutionary potential of parasites. This is contrary to predictions for immigration from cold spots in which parasites are present but coevolutionary interactions are weaker, and under such conditions immigration would be expected to weaken coevolutionary interactions in the hot spot (Gomulkiewicz et al. 2000).

Our results are also relevant to considerations of local adaptation. Parasite migration has been shown to increase sympatric infectivity of parasites both theoretically (Gandon et al. 1996; Gandon and Michalakis 2002) and empirically (Morgan et al.

2005). However, a recent model suggests that patterns may be more complex in heterogeneous coevolving metapopulations (Nuismer 2006). Here, we show that for a wide range of rates, immigration of hosts from unparasitized populations can also increase the sympatric performance of parasites, particularly when considered over coevolutionary timescales. This suggests that the conditions favoring local adaptation of parasites in host-parasite metapopulations may be broader when some patches that contribute host migrants are free of the parasite, and may help to explain unexpected parasite local adaptation despite higher relative host migration observed in field studies (Ganz and Washburn 2006).

The generality of the coevolutionary patterns observed in this study may be somewhat limited to host-parasite systems that undergo predominantly directional selection (Buckling and Rainey 2002a, b; Brockhurst et al. 2004). Such systems include certain plant pathogen interactions (Burdon and Thrall 1999; Thrall and Burdon 2003) that broadly comply with a multilocus gene-for-gene model of coevolutionary interaction, which allows for the evolution of generalist host and parasite types (Damgaard 1999; Sasaki 2000; Thrall and Burdon 2002). Under such conditions naïve immigrant hosts are likely to be susceptible to local parasites. However, this is unlikely to be the case in host-parasite systems in which infection relies upon highly specific matching of host and parasite genotypes and in which selection is predominantly fluctuating (i.e., matching alleles interactions [Agrawal and Lively 2002; Agrawal and Lively 2003]). Here, the impact of naïve host immigrants is likely to depend upon the degree of parasite specificity and whether hosts or parasites are currently locally adapted (Gandon 2002; Gandon and Michalakis 2002; Morgan et al. 2005; Nuismer 2006).

Our results have potentially important implications for health and agriculture. For a wide range of rates, the migration of people, livestock, insects, and crops into areas with endemic diseases to which they have not previously been exposed but are susceptible is likely to significantly enhance the evolutionary potential of endemic parasites. Such effects will be to some extent case specific (see the previous paragraph) and will depend on, among other things, the virulence and resistance mechanisms and the relative generation times of host and parasite. However, where parasite evolutionary potential is enhanced this is likely to lead, not only to severe outbreaks of disease, but also to altered patterns of local adaptation and intensification of antagonistic coevolutionary interactions.

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