Migration highways and migration barriers created by host-parasite interactions

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Abstract

Coevolving parasites may play a key role in host migration and population structure. Using coevolving bacteria and viruses, we test general hypotheses as to how coevolving parasites affect the success of passive host migration between habitats that can support different intensities of host-parasite interactions. First, we show that parasites aid migration from areas of intense to weak coevolutionary interactions and impede migration in the opposite direction, as a result of intraspecific apparent competition mediated via parasites. Second, when habitats show qualitative difference such that some environments support parasite persistence while others do not, different population regulation forces (either parasitism or competitive exclusion) will reduce the success of migration in both directions. Our study shows that coevolution with parasites can predictably homogenizes or isolates host populations, depending on heterogeneity of abiotic conditions, with the second scenario constituting a novel type of "isolation by adaptation".

Keywords: adaptation, coevolution, consumer-resource interactions, experimental evolution, geographic mosaic of coevolution, local adaptation, isolation by adaptation

INTRODUCTION

Migration is one of the principal forces to drive population dynamics and evolution (Wright 1943; Holt & Gomulkiewicz 1997; Lenormand 2002; Morjan & Rieseberg 2004; Morgan *et al.* 2005; Forde *et al.* 2007; Savolainen *et al.* 2007), hence understanding the likelihood of successful migration is crucial. Coevolution with parasites has long been recognized as an important factor determining the success of migration of host populations. This, for example, has been a generally accepted explanation for the successful colonization of the New World by Eurasian civilizations, where the resident human hosts were less well-defended against parasites co-dispersing with the immigrant hosts (Diamond 1999). However, a general understanding is still lacking about the ecological contexts in which coevolving parasites help or hinder host migration. Here we focus on how environmental heterogeneity in abiotic conditions can influence parasite-mediated host migration success.

In the absence of parasites, adaptation to heterogeneous environments may lead to local adaptation; and this can limit the colonization of subsequent immigrants and thus reduce gene flow, namely "isolation by adaptation" (Thompson 2005; Orsini *et al.* 2013). In principle, coevolution with parasites can also result in host or parasite local adaptation (i.e., higher fitness on local versus foreign populations of the interacting species) which itself can predictably affect immigration success. While abiotic environmental heterogeneity can sometimes enhance such host-parasite specialization (Lopez-Pascua *et al.* 2012; Gorter *et al.* 2016), it may also create spatial variation in the strength of coevolutionary interactions, with some habitats containing universally more resistant hosts and more infectious parasites than others (Thompson 1994; Hochberg & van Baalen

1998; Thompson 1999; Gomulkiewicz *et al.* 2000; Thrall & Burdon 2003; Thompson 2005; Laine 2008; Wolinska & King 2009). Nonetheless, rather than the resultant between-habitat variation in local adaptation making it harder to predict the average effect of parasites on immigration success, we suggest that environmental heterogeneity will actually have highly predictable effects on immigration success.

First, a host-parasite system may show quantitative variation in the intensity of coevolutionary interactions among habitats. For example, hosts and parasites often evolve strong defences and counter-defences, respectively, in high-productivity habitats due to large population sizes and thus intense selection for defences as well as rapid supply of genetic variation (Hochberg & van Baalen 1998; Forde *et al.* 2004; Lopez-Pascua & Buckling 2008; Best *et al.* 2010). It is suggested that immigrants from such "coevolutionary hot spots" are then more likely to invade "cold spots" where coevolution is weaker, but migration in the opposite direction may be impeded (Thompson 1999; Thompson 2005; Forde *et al.* 2007; Lopez-Pascua *et al.* 2010). Under this scenario, parasitism is the predominate population regulation force for the hosts and is likely to augment the effect of dispersal in homogenizing dynamics of multiple local populations, via parasite-mediated intraspecific competition (Holt & Barfield 2009; Ricklefs 2010). Previous studies have provided indirect support by tracking the spread of host resistance and parasite infectivity traits, but the fate of immigrants was not directly examined (Forde *et al.* 2007; Lopez-Pascua *et al.* 2010). Second, environmental heterogeneity may also cause qualitative differences in host-parasite interactions, e.g., when there is a mismatch between hosts and parasites in requirements for abiotic environmental conditions and thus parasites survive only in a portion of habitats occupied by their hosts (Fels & Kaltz

2006; Laine 2007; King *et al.* 2009; Zhang & Buckling 2011). In this situation, parasites may impede host migration in both directions. Hosts from parasite-present habitats will be competitively inferior when introduced into parasite-free environments as they carry costly, unnecessary, defence traits (Bergelson & Purrington 1996; Bohannan & Lenski 2000; Buckling *et al.* 2006); whereas hosts from parasite-free environments will suffer high mortality due to parasitism when invading a parasite-present habitat. Here the presence of parasite species increase the niche dimensionality for the host species, and their response to the abiotic environment results in distinct population regulation forces (parasitism versus competition) for the hosts in different habitats. No previous work has tested this hypothesis.

We tested these ideas using experimental populations of the bacterium *Pseudomonas fluorescens* SBW25 and its lytic bacteriophage virus SBW25Φ2. This host-parasite system can undergo intensive antagonistic coevolution between resistance and infectivity traits under certain, benign, laboratory environments (Buckling & Rainey 2002), with increased resistance and infectivity associated with growth rate costs (Buckling *et al.* 2006; Poullain *et al.* 2008). Low temperature can limit the rate of bacterial (and thus phage) growth and constitutes a low-productivity environment (Gorter *et al.* 2016). High temperature, within a certain range, can prevent phage growth while having little impact on bacterial growth, and therefore creates a parasite-free environment (Zhang & Buckling 2011). In the present study we first allowed just bacteria or bacteria and phages to (co)evolve in different temperature environments for a period of time without migration, and then imposed experimental dispersal on those populations and examined the success of host immigrants. With this experimental approach we were able

to unambiguously study how coevolution with parasites affects host immigration success in heterogeneous environments.

METHODS

Strains and culture conditions

This study used two bacterial strains, *Pseudomonas fluorescens* SBW25 (Rainey & Bailey 1996), and a modified variant SBW25EeZY6KX (Bailey *et al.* 1995), and one bacteriophage strain, SBW25Φ2 (Buckling & Rainey 2002). SBW25EeZY6KX contains two constitutively expressed marker gene cassettes, one consisting of genes encoding kanamycin resistance and catechol 2,3-dioxygenase and the other consisting of lacZY genes enabling the utilization of lactose. SBW25EeZY6KX shows no detectable difference in fitness from the wild-type SBW25 strain in the nutrient medium (M9KB) used in the present study (Fig. S1), but is resistant to the antibiotic kanamycin and has a blue color when grown as colonies on agar plates supplemented with X-gal (SBW25 colonies being yellow).

Bacteria and phages were grown in static microcosms of 6 mL of M9KB medium (M9 salt solution supplemented with 10 g L^{-1} glycerol and 20 g L^{-1} proteose peptone no. 3) in 30 mL glass tubes with loose lids. We considered the 25 °C habitat as a benign environment that supports strong coevolution between bacterial resistance and phage infectivity; the 15 °C habitat is a low-productivity habitat where coevolution is weak; and the 31 °C habitat soon becomes a parasite-free environment, hence there is greatly reduced selection for resistance. The assumptions were shown to be true by comparing bacterial resistance/phage infectivity between these environments (see Results).

147

148 **Evolution/coevolution experiment and measurement of resistance and infectivity**

149 The aim of the study was to examine how coevolution with phages affects the success of
150 bacterial migration between the 25 and 15 °C habitats (migration of either 25-to-15 or 15-
151 to-25 direction), and between the 25 and 31 °C habitats (migration of 25-to-31 or 31-to-
152 25 direction). Forty-eight "metapopulations" were assembled, 24 of which were grown
153 with bacteria only (evolution lines) and the other 24 with both bacteria and phages
154 (coevolution lines). Each metapopulation consisted of one "source" and one "recipient"
155 microcosm. For example, 12 metapopulations were assigned to 25-to-15 migration
156 treatment, six of which were grown with bacteria only and the other six with both
157 bacteria and phages; and source microcosms of these metapopulations were incubated at
158 25 °C, and recipient ones, 15 °C.

159 Every microcosm was initially inoculated with ~10^8 bacterial cells, with or
160 without ~10^5 phage particles. All source microcosms were inoculated with bacterial
161 strain SBW25EeZY6KX, and recipient, SBW25. Then cultures were propagated for 6
162 serial transfers, one transfer every 48 h. At each transfer, 60 μL (1%) of culture from
163 each microcosm was transferred to fresh media. During the 6 transfers of the
164 evolution/coevolution experiment, all microcosms evolved independently and there was
165 no dispersal between the source and recipient microcosms.

166 At transfer 6, samples of bacteria and phages were drawn from the coevolution
167 lines (where both bacteria and phage were inoculated). Dilutions of the 6-transfer-old
168 cultures were spread onto agar plates, and incubated for 48 h at 25 °C, to obtain
169 independent bacterial colonies. Phage samples were isolated from cultures by mixing

100 µL of chloroform and 900 µL of each culture, which was then vortexed to lyse the bacterial cells, and centrifuged at 15 800 g for 2 min to pellet the bacterial debris, leaving a suspension of phages in the supernatant. Phage density measurement (spotting phage dilutions onto soft agar plates containing the ancestral bacterial cells and counting plaque forming units after 24 h incubation at 25 °C) suggested that all phage lines at 25 and 15 °C survived until transfer 6, and all phage lines at 31 °C went extinct. Bacterial resistance/phage infectivity was compared between the source and recipient microcosms within each metapopulations. This was achieved by a reciprocal challenge test: resistance of bacteria from the source and recipient microcosms was estimated against phages from both source and recipient microcosms. To measure the resistance a bacterial population against a given phage population, we streaked suspensions of 20 independent bacterial colonies across a line of phage (20 µL) that had previously been streaked and dried on a M9KB agar plate. A colony was scored as resistant if there was no sign of growth inhibition by the phage after 24 h incubation (at 25 °C), otherwise it was susceptible. Resistance of the bacterial population was defined as the proportion of resistant colonies. Assays of bacterial resistance to phages from microcosms maintained at 31 °C were not performed due to phage extinction. Note that bacterial resistance in the evolution lines (metapopulations inoculated with bacteria only), measured in the same way against the ancestral phage, was non-detectable.

Dispersal experiment

Immediately after transferring the 6-transfer-old cultures to fresh microcosms, we moved 5% of culture from each source microcosm to its corresponding recipient microcosm

193 (phages and bacteria dispersed simultaneously in the coevolution metapopulations). The
194 success of immigrant bacteria in recipient microcosms during transfer 7 was estimated as
195 follows. Initial and final densities of immigrant (SBW25EeZY6KX) and resident
196 (SBW25) bacteria were measured by plating diluted cultures onto M9KB agar plates
197 (10^6-fold dilutions plated onto agar plates with X-gal, on which both SBW25 that showed
198 a yellow color and SBW25EeZY6KX that was blue could grow; and 10^4-fold dilutions
199 plated onto agar plates with both X-gal and kanamycin, where only SBW25EeZY6KX
200 could grow), and counting the number of colony forming units (CFUs) after 48 h
201 incubation at 25 °C. For each metapopulation, we estimated the success of migration in
202 terms of per capita growth rate of immigrant bacteria relative to residents in the recipient
203 microcosm, as a "selection-rate constant" (Lenski *et al.* 1991). A Malthusian parameter
204 was calculated for both immigrant and resident populations, $m = \log_{10}(N_f/N_0)/(1 \text{ transfer})$,
205 where N_0 and N_f were the initial and final densities. The selection-rate constant was $r =$
206 $m_{\text{immigrant}} - m_{\text{resident}}$. An r value of zero suggests no difference in growth rate between
207 immigrants and residents; and $r > 0$ indicates an advantage of immigrants in population
208 growth (more specifically, an r value of 1 indicates a 10-fold increase in the ratio of
209 immigrant versus resident abundances), while $r < 0$ suggests a failure of immigrants to
210 invade the resident population. Note that in this study, as in many microbial systems,
211 dispersal was passive, and random in terms of the composition of dispersing individuals
212 (which were randomly drawn from a source population), while the estimate of migration
213 success was an average measure on the population level. We cannot rule out a possibility
214 that a positive value of migration success was contributed to by only a portion of
215 immigrant genotypes, with other genotypes failing to invade the recipient habitats; in this

case, our "selection-rate constant" measure may become an underestimate for the invasion ability of the specific genotypes that did colonize the recipient habitats.

Data analyses

Data were analyzed in the R environment. Bacterial resistance data were arcsine transformed before analysis. Resistance data from metapopulations of 25-to15 or 15-to-25 migration direction were analyzed using mixed-effect linear models, with the type of origin microcosm (source versus recipient) of the tested bacteria and that of the tested phages as two categorical explanatory variables and metapopulation ID as a random factor. Resistance data from metapopulations of 25-to-31 or 31-to-25 migration direction, which involved measurement of resistance of bacteria against phages from the 25, but not the 31 °C, microcosms (as phages went extinct at 31 °C) were analyzed using paired Wilcoxon signed-rank test (parametric analyses were not appropriate due to violation of the assumptions of equal variances and normal error distributions). Selection-rate constant data were square-root transformed while preserving the original positive or negative signs, and analyzed using ANOVA, with migration direction and the presence of phages as two categorical explanatory variables. For coevolution metapopulations under migration between the 25 and 15 °C habitats, we also calculated the difference in bacterial resistance between source and recipient microcosms (averaged across two types of tested phages, i.e., that from source or recipient microcosms) at transfer 6, and tested for its correlation with the success of bacterial migration in the dispersal experiment (transfer 7).

RESULTS

Migration between the 25 and 15 °C habitats

Measurement of bacterial resistance and phage infectivity for the coevolution lines at transfer 6 (prior to the dispersal experiment) confirmed that the 25 °C environment was a coevolutionary hot spot relative to the 15 °C environment. For metapopulations assigned to the 25-to-15 dispersal treatment, the source (25 °C) microcosms had higher bacterial resistance and higher phage infectivity relative to the recipient (15 °C) microcosms: bacterial resistance against phages was higher when the tested bacteria were from the source (25 °C) microcosms, but lower when the tested phages were from the source (25 °C) microcosms (mixed-effect linear model, bacteria, $F_{1,15} = 7.24$, $P = 0.017$; phages, $F_{1,15} = 6.51$, $P = 0.022$; bacteria × phages interaction, $F_{1,15} = 0.697$, $P = 0.420$; Fig. 1a). For metapopulations assigned to the 15-to-25 dispersal treatment, the source (15 °C) microcosms had lower bacterial resistance relative to the recipient (25 °C) microcosms, and phage infectivity did not show a significant difference between the two environments (bacteria, $F_{1,15} = 16.01$, $P = 0.001$; phage, $F_{1,15} = 1.91$, $P = 0.187$; bacteria × phages interaction, $F_{1,15} = 0.363$, $P = 0.556$; Fig. 1b).

During the dispersal experiment, the presence of coevolving phages increased the success of bacterial migration from the 25 to 15 °C habitats, but decreased migration success in the opposite direction (ANOVA analysis of selection-rate constant, dispersal direction, $F_{1,20} = 13.0$, $P = 0.002$; phage, $F_{1,20} = 0.004$, $P = 0.951$; direction × phage interaction, $F_{1,20} = 8.83$, $P = 0.008$; Fig. 2). Specifically, in metapopulations without phages, immigrant bacteria did not show a significant difference in growth rate from resident bacteria in either dispersal direction (selection-rate constant not different from

zero, one-sample t test, 25-to-15 dispersal, $t = 1.46$, df = 5, $P = 0.205$; 15-to-25 dispersal, $t = 0.526$, df = 5, $P = 0.622$). However, in the presence of coevolving phages, immigrant bacteria from the 25 °C microcosms had a population growth advantage when introduced into the 15 °C microcosms (selection-rate constant larger than zero, $t = 3.07$, df = 5, $P = 0.028$), while 15 °C migrants failed to invade 25 °C populations (selection-rate constant almost significantly smaller than zero, $t = -2.27$, df = 5, $P = 0.073$). Across all the coevolution metapopulations, the relative growth rate of immigrant bacteria in the recipient microcosms was positively correlated with the difference between source and recipient microcosms in bacterial resistance (Pearson's correlation test, $r = 0.833$, df = 10, $P < 0.001$; Fig. S2).

Migration between the 31 and 25°C habitats

As hypothesized, bacterial resistance evolved to a much lower level in the 31 °C compared with the 25 °C environment. As phages went extinct in all 31 °C microcosms, measurement of resistance involved challenging bacteria from both the 25 and 31 °C microcosms against phages from the 25 °C microcosms only. For metapopulations assigned to either 25-to-31 or 31-to-25 dispersal treatment, bacteria from the 31 °C microcosms showed non-detectable resistance, which was much lower than the resistance of bacteria from the 25 °C microcosms (paired Wilcoxon test, 25-to-31 dispersal metapopulations, $P = 0.036$; 31-to-25 dispersal metapopulations, $P = 0.031$; Fig. 3).

The success of bacterial migration in either direction was significantly reduced in metapopulations with phages, and the impact of phages was strongest for the 31-to-25 dispersal (ANOVA, dispersal direction, $F_{1,20} = 37.4$, $P < 0.001$; phage, $F_{1,20} = 49.8$, $P <$

285 0.001; direction × phage interaction, $F_{1,20} = 7.12$, $P = 0.015$; Fig. 4). In the absence of
286 phages, immigrant bacteria from the 25 °C microcosms showed no difference in growth
287 rate from the residents when introduced into the 31 °C microcosms (selection-rate
288 constant not different from zero, $t = -1.19$, df = 5, $P = 0.289$), while immigrant bacteria
289 from the 31 °C microcosms showed lower growth rates relative to residents when
290 introduced into the 25 °C microcosms (selection-rate constant smaller than zero, $t = -$
291 10.41, df = 5, $P < 0.001$). In metapopulations with phages, immigrant bacteria had lower
292 growth rates than residents in both dispersal directions (selection-rate constant smaller
293 than zero, 25-to-31 dispersal, $t = -8.52$, df = 5, $P < 0.001$; 31-to-25 dispersal, $t = -16.85$,
294 df = 5, $P < 0.001$).
295
296 **DISCUSSION**
297 While the effects of migration on host-parasite coevolution have been extensively studied
298 (Lively 1999; Nuismer 1999; Gandon & Michalakis 2002; Forde *et al.* 2004; Morgan *et*
299 *al.* 2005; Kerr *et al.* 2006; Forde *et al.* 2007; Morgan *et al.* 2007; Vigneux *et al.* 2008;
300 Gandon & Nuismer 2009; Lopez-Pascua *et al.* 2010), the question of how the success of
301 migration is driven by host-parasite coevolution has received less attention. Here our
302 experiments with a bacterium-phage system suggest that parasites may predictably
303 increase or decrease the success of host migration, depending on how host-parasite
304 interactions vary across abiotic environments. The present study examined how host-
305 parasite interactions affect the success of passive migration, and not the evolution of
306 dispersal rates. However, our findings do provide insights into how parasite-imposed

307 selection might act on the evolution of migration rates, by identifying the conditions
308 under which migration is helped or hindered.

309 While adaptation to the abiotic environment affected migration in some contexts
310 (adaptation to 31 °C led to a reduction in fitness at 25 °C, but not vice versa, for unknown
311 reasons), environment-dependent host-parasite coevolution showed much more
312 pronounced effects. Our results confirmed that in environments where parasites are a
313 ubiquitous selection pressure (the linked 25 °C and 15 °C microcosms), intraspecific
314 apparent competition could lead to highly asymmetrical migration between habitats that
315 show quantitative variation in the extent of evolved resistance and infectivity.
316 Specifically, the presence of coevolving phage parasites increased the success of bacterial
317 migration from the 25 to 15 °C habitats, and decreased the success of migration in the
318 opposite direction, while environmental conditions had no significant impact on
319 immigration success in the absence of parasites (Fig. 2); this effect can well be explained
320 by the difference in resistance between immigrant and resident bacteria (Fig. 1; Fig. S2).
321 Possible explanations for the differences in evolved resistance and infectivity include
322 greater population sizes (Fig. S1) or mutation rates (Gillooly *et al.* 2005) at high
323 temperatures resulting in an increased supply of genetic variation and thus faster
324 coevolution (Gorter *et al.* 2016), or elevated costs of resistance in low-productivity (here,
325 low-temperature) environments limiting arms-race-like coevolution and favoring
326 fluctuating selection dynamics (Hall *et al.* 2011; Lopez-Pascua *et al.* 2014).

327 The scenario discussed above (intraspecific apparent competition leading to
328 highly asymmetrical migration between habitats) has important implications for our
329 understanding of synchronization of population dynamics in changing environments.

Populations of consumer-resource species interactions are more prone to the synchronizing effects of dispersal than those of single species, as predicted by ecological models of spatial coupling of predation effects (Vasseur & Fox 2009; Vogwill *et al.* 2009; Duncan *et al.* 2015). Our results here imply that genetic homogenization of populations may also be involved in population synchronization if coevolution takes place between the interacting species. It is unclear whether this will lead to distinct predictions for the long-term population dynamics.

Our second key finding is that, when habitats show qualitative variation in host-parasite interactions such that some environments support parasite persistence while others do not, migration in either direction can be reduced by coevolutionary interactions with parasites. This was shown by our treatments involving bacterial migration between the 25 and 31 °C environments. The 31°C treatment prevented phage growth but had little impact on bacterial growth, creating a parasite-free environment where selection for host resistance was lacking. Immigrants moving from this parasite-free environment to the habitat with parasites were selected against because of the lack of resistance. Meanwhile, resistant bacteria that migrated into parasite-free habitats were also selected against because of fitness costs of resistance, which has been well-documented in this system (Buckling *et al.* 2006; Lopez-Pascua & Buckling 2008).

When populations evolve under divergent selection in different habitats, local adaptation may lead to population diversification, with reduced gene flow (Thompson 2005; Orsini *et al.* 2013). Such "isolation by adaptation" occurred between parasite-present and parasite-free environments in our experiment. This is because the hosts were under distinct selection forces across the two types of environments (parasitism versus

353 competition), which resulted from the response of the parasites to heterogeneous abiotic
354 environments. Therefore, geographic structure of parasites set by physical factors may
355 result in population isolation in hosts, with a potential for promoting population
356 diversification and speciation.

357 Our experimental design involved simultaneous migration of hosts and parasites,
358 a situation that is likely to be the norm in nature. However, if hosts migrated alone, it is
359 likely that immigrants would be disfavored in all contexts. First, where habitats differ
360 quantitatively in the strength of coevolutionary interactions, the success of the intensely
361 coevolving host immigrants is a direct consequence of the presence of their intensively
362 coevolved parasites. In the absence of these parasites, it is likely that levels of evolved
363 defences would be too costly in the evolutionary cold spots. Second, in the context
364 where environments showed qualitative variation in parasite persistence, simultaneous
365 host/parasite migration is effectively the same as host-only migration.

366 In summary, our study shows that coevolving parasites may have diverse
367 predictable effects on host migration in heterogeneous environments, and thus may
368 promote population homogenization in some environmental contexts, but population
369 isolation under other conditions. This is because the responses to abiotic environments
370 determine how the presence of parasites can alter the heterogeneity among habitats for
371 hosts in terms of selection agents. Therefore the consequences of the environmental
372 dependence in host-parasite coevolutionary interactions for metapopulation dynamics and
373 between-population diversity deserve more research, particularly in the context that
374 migration and adaptation are needed for species persistence under climate change.
375

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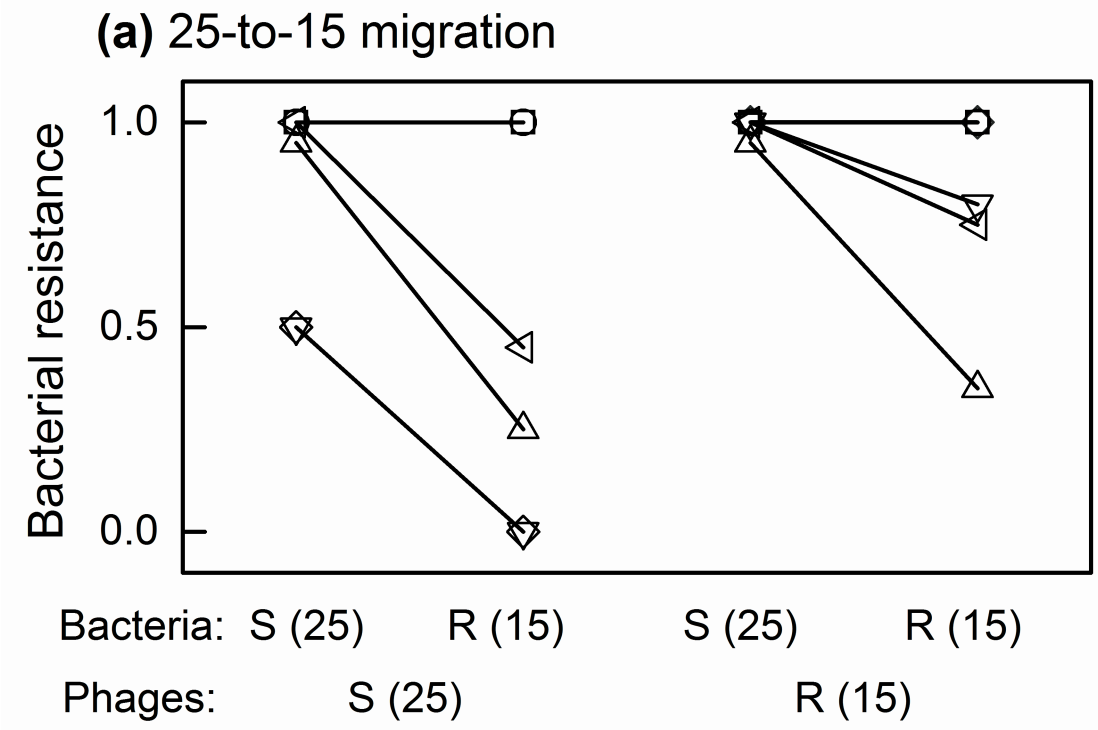
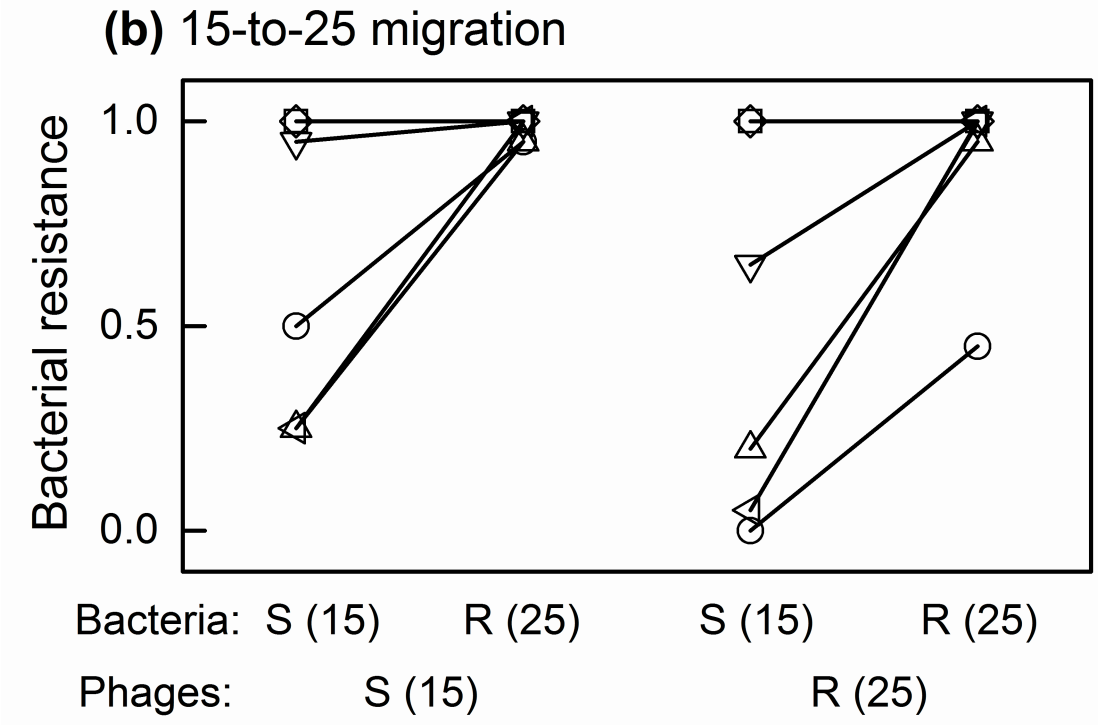
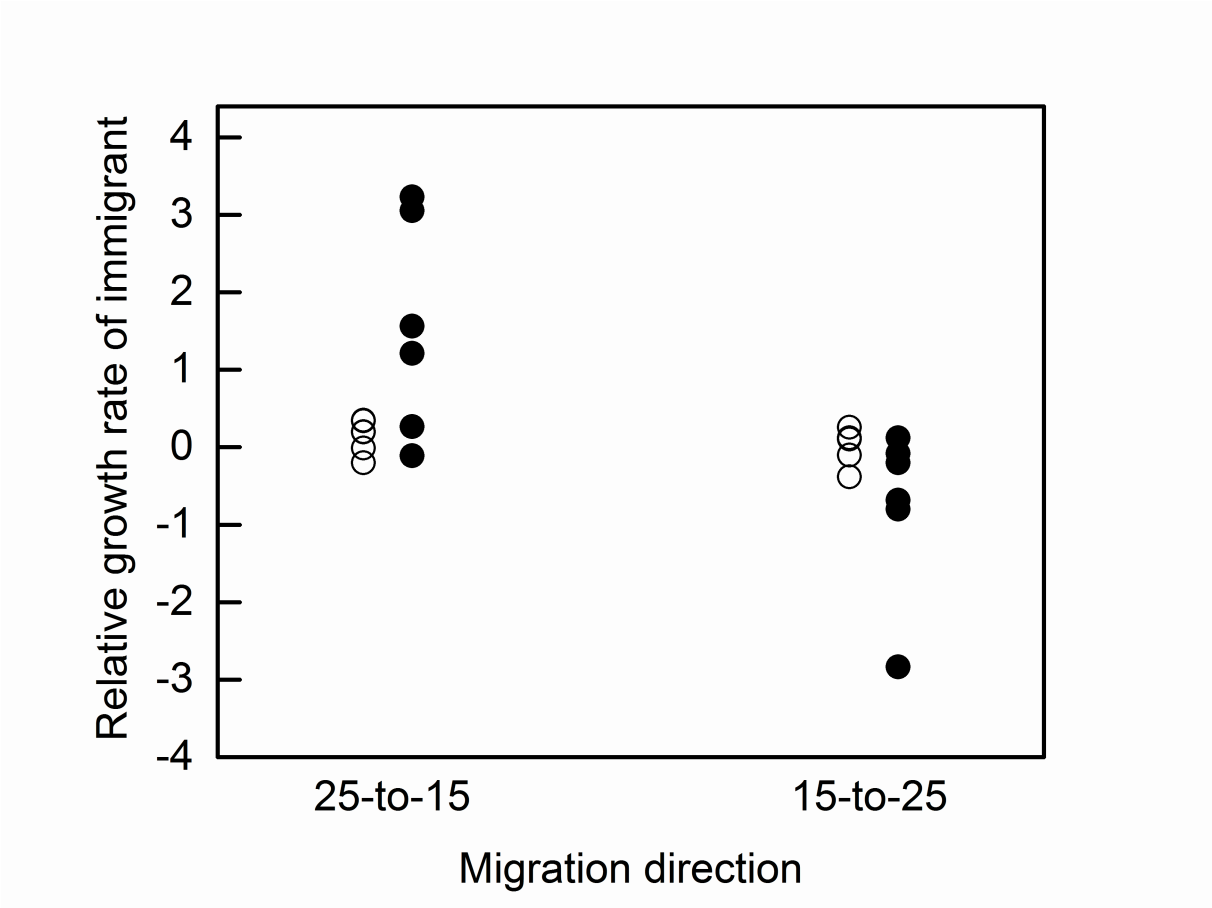
561 **SUPPORTING INFORMATION**
562 Additional Supporting Information may be downloaded via the online version of this
563 article at XXX.


(a) 25-to-15 migration
Bacterial resistance
1.0
0.5
0.0
Bacteria: S (25) R (15) S (25) R (15)
Phages: S (25) R (15)


(b) 15-to-25 migration
Bacterial resistance
1.0
0.5
0.0
Bacteria: S (15) R (25) S (15) R (25)
Phages: S (15) R (25)

564


Relative growth rate of immigrant
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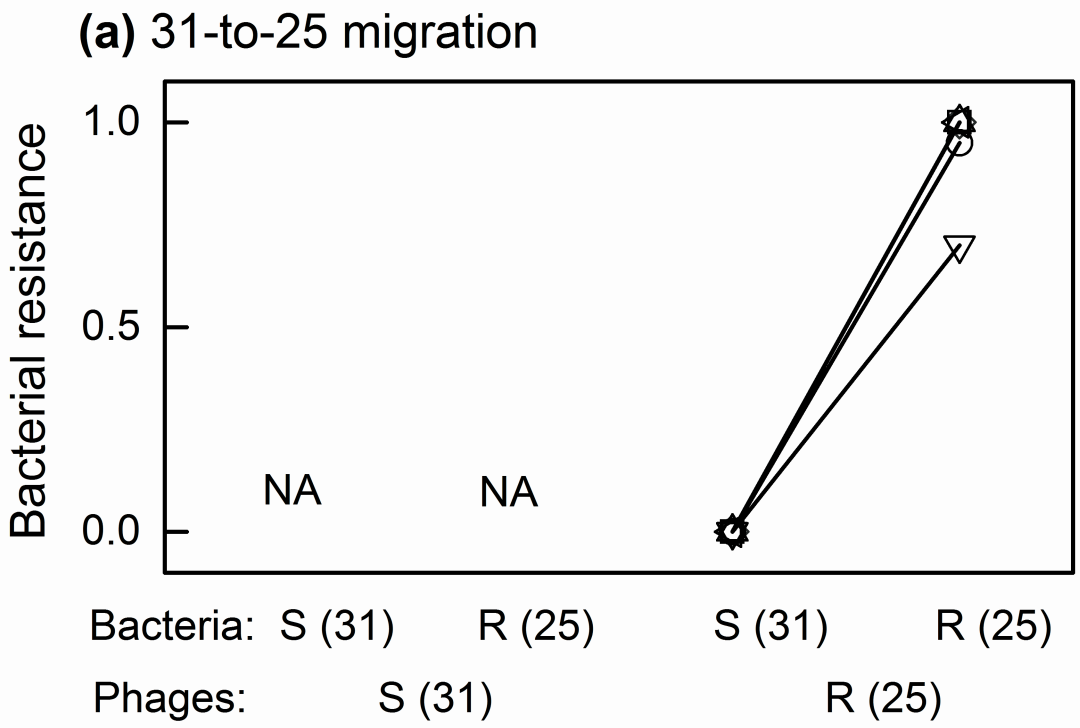
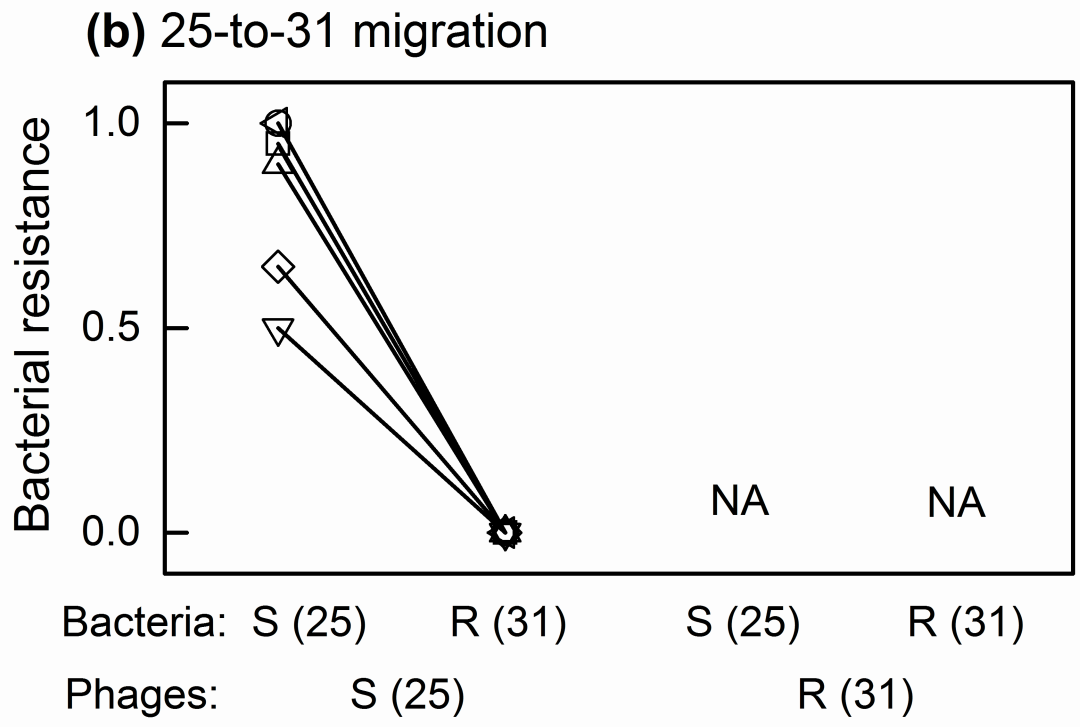
3
2
1
0
-1
-2
-3
-4
25-to-15
15-to-25
Migration direction

565


(a) 31-to-25 migration
Bacterial resistance
1.0
0.5
0.0
NA
NA
Bacteria: S (31) R (25) S (31) R (25)
Phages: S (31) R (25)


(b) 25-to-31 migration
Bacterial resistance
1.0
0.5
0.0
NA
NA
Bacteria: S (25) R (31) S (25) R (31)
Phages: S (25) R (31)

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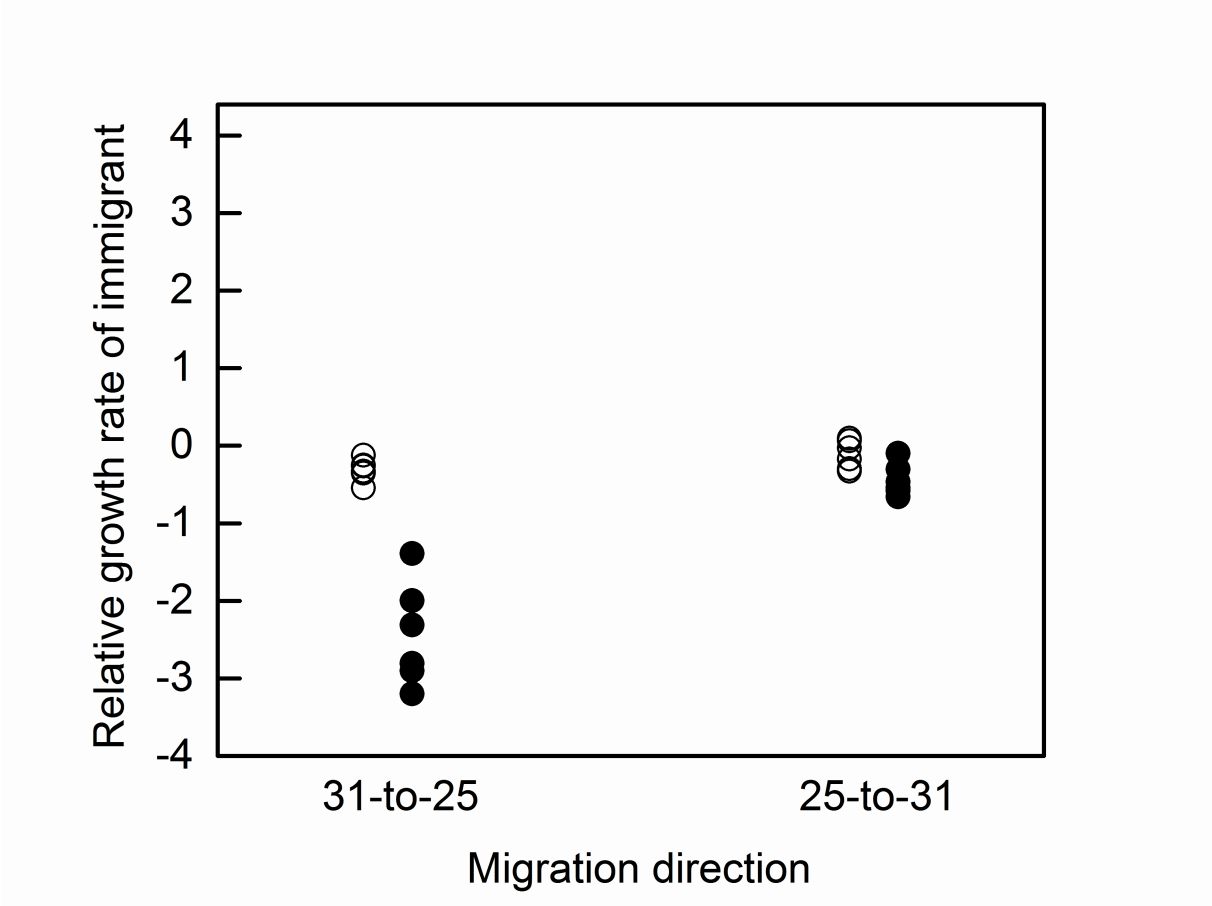
Relative growth rate of immigrant
4
3
2
1
0
-1
-2
-3
-4
31-to-25
25-to-31
Migration direction

568
569

570 **Figure legend**

571

572 Fig. 1 Resistance of bacteria to phages in metapopulations assigned to 25-to-15 (a) and
573 15-to-25 (b) coevolution treatments. Numbers in x-axis titles indicate culture
574 environment (25 or 15 °C) where the tested bacteria and tested phages were sampled
575 from; "S" represents source microcosms, and "R" recipient microcosms. Within every
576 panel each symbol indicates tests from one individual metapopulation (six replicate
577 metapopulations per treatment).

578

579 Fig. 2 Growth rate of immigrant bacteria relative to residents in metapopulations of the
580 25 and 15 °C habitats, in the absence (open circles) or presence (filled circles) of
581 coevolving phages.

582

583 Fig. 3 Resistance of bacteria to phages in metapopulations assigned to 31-to-25 (a) and
584 25-to-31 (b) coevolution treatments. Numbers in x-axis titles indicate culture
585 environment (31 or 25 °C) where the tested bacteria and tested phages were sampled
586 from; "S" represents source microcosms, and "R", recipient microcosms. Note that data
587 for resistance against phages from the 31 °C habitat are missing due to phage extinction.

588

589 Fig. 4 Growth rate of immigrant bacteria relative to residents in metapopulations of the
590 31 and 25 °C habitats, in the absence (open circles) or presence (filled circles) of
591 coevolving phages.

592