LETTERS

The effect of migration on local adaptation in a coevolving host–parasite system

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Antagonistic coevolution between hosts and parasites in spatially structured populations can result in local adaptation of parasites[1–5]; that is, the greater infectivity of local parasites than foreign parasites on local hosts[1]. Such parasite specialization on local hosts has implications for human health and agriculture. By contrast with classic single-species population-genetic models[6,7], theory indicates that parasite migration between subpopulations might increase parasite local adaptation, as long as migration does not completely homogenize populations[8–11]. To test this hypothesis we developed a system-specific mathematical model and then coevolved replicate populations of the bacterium *Pseudomonas fluorescens* and a parasitic bacteriophage with parasite only, with host only or with no migration. Here we show that patterns of local adaptation have considerable temporal and spatial variation and that, in the absence of migration, parasites tend to be locally maladapted. However, in accord with our model, parasite migration results in parasite local adaptation, but host migration alone has no significant effect.

Conventional wisdom holds that parasites have an evolutionary advantage when antagonistically coevolving with their hosts; they are therefore expected to be locally adapted to their hosts, rather than vice versa[2]. Several studies support this prediction[12–15], but others find no evidence of local adaptation, and some even find parasite local maladaptation[16–19]. Recent theoretical work may be able to explain these varied empirical results. The species that can adapt the fastest is most likely to be locally adapted, and speed of adaptation can be limited by the amount of within-population genetic variation[8–11]. An important source of genetic variation is migration between populations, because it potentially allows the introduction of genes beneficial at a particular point in space and time[8–11]. If we assume that migration rates are not so high as to homogenize populations completely, and other factors that might influence local adaptation are equal between hosts and parasites, parasites are predicted to be locally adapted if they migrate more than their hosts, whereas hosts are predicted to be locally adapted if they migrate more than their parasites[8–11].

Recent experimental studies have demonstrated that migration can alter coevolutionary dynamics[20,21], but whether relative migration rates of hosts and parasites are a significant determinant of local adaptation has yet to be empirically investigated. We examined the role of differential migration rates of hosts and parasites on parasite local adaptation with the use of the aerobic Gram-negative bacterium *Pseudomonas fluorescens* as the host and an associated lytic DNA phage SBW25ϕ2 as the parasite[19]. Large population sizes and short generation times favour rapid evolution[22], and the antagonistic nature of the interaction (phages must lyse the bacteria to release progeny virions) results in sustained coevolution between bacterial resistance and phage infectivity[19], as well as favouring the evolution of local adaptation[9]. Detecting coevolutionary change is made possible by the ability to store populations in 'suspended animation' in a freezer, which allows interactions to be directly measured across space and time[19].

Throughout this study, we quantify parasite local adaptation as the performance of local parasites minus that of the average performance of foreign parasites ('local versus foreign')[1], where parasite performance is measured as the proportion of bacterial clones sensitive to phage. A positive value indicates parasite local adaptation and a negative value parasite local maladaptation. Note that parasite local maladaptation does not necessarily imply host local adaptation, when considering a single focal population. However, when considering data averaged across all populations, mean parasite local adaptation equates with mean host local maladaptation (see Supplementary Information).

We performed simulation studies to show that theoretical predictions about the impact of migration on local adaptation hold true using parameter values that approximate our biological system and experimental design (see Supplementary Information for more details). A key parameter in determining coevolutionary dynamics is the specificity of the interaction between host and parasite[23,24]. Because we do not know the underlying model of specificity governing the interaction between the bacteria and the phage, we used a general model of coevolution that allowed us to explore different circumstances[23] (see Supplementary Methods). At one extreme of this model the different host and parasite genotypes

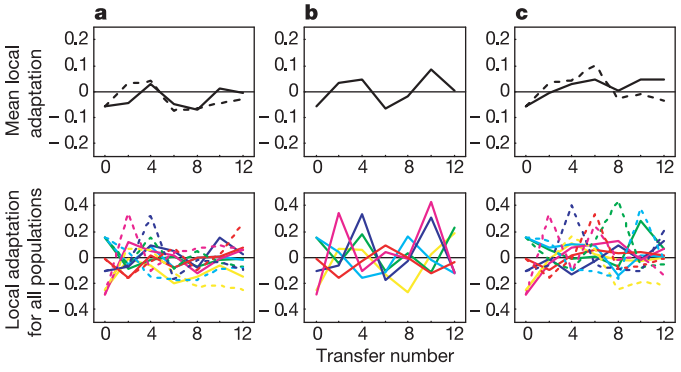

Figure 1 | The simulated effect of differential migration rates on local adaptation. Parasite local adaptation is shown through time for individual populations (bottom) and for the average over the different populations (top). **a**, Host migration only; **b**, no migration; **c**, parasite migration only. In **a** and **c**, solid lines show 10% migration, dashed lines 0.1% migration. Other parameter values are as follows: $\mu_h = \mu_p = 10^{-6}$, $c = k = 0.05$, $\sigma_h = 0.9$, $\sigma_p = 0.5$, $a = 0.5$. See Supplementary Information for model details. A positive value indicates that the parasite is locally adapted, and a negative value that the parasite is locally maladapted.

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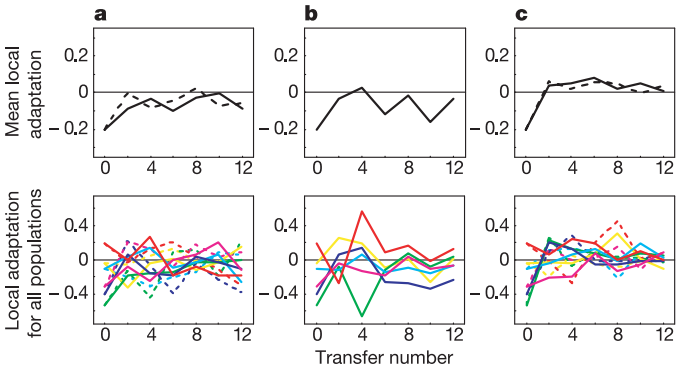

Figure 2 | The effect of differential migration rates on local adaptation. Parasite local adaptation is shown through time for individual populations (bottom) and for the average over the different populations (top). **a**, Host migration only; **b**, no migration; **c**, parasite migration only. In **a** and **c**, solid lines show 10% migration, dashed lines 0.1% migration.

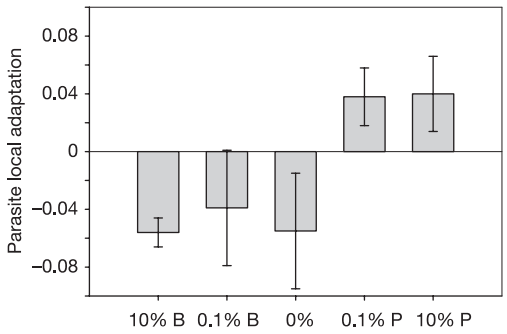

Figure 3 | Mean local adaptation of parasites. Data are averaged over six populations and six time points (T_2, T_4, T_6, T_8, T_{10} and T_{12}) for different migration treatments. Results are means ± s.e.m. across populations. P, phage migration; B, bacterial migration.

have identical levels of specificity in resistance and infectivity, respectively ('matching alleles' model, when $a = 0$)[23]. At the other extreme, the model allows variable levels of specificity among the different host and parasite genotypes, allowing for the evolution of generalists ('gene for gene' model, when $a = 1$)[23]. Under a pure 'gene for gene' model the spread of universally resistant hosts and universally infective parasites prevents the emergence of local adaptation[9]. However, our simulations show that coevolution where generalists can evolve (that is, $a > 0$), as occurs in our experimental system[19], can result in local adaptation for a broad range of parameter values (provided that $a < 1$; see Supplementary Fig. S2).

Figure 1 shows an example of a simulation where parasites are locally maladapted in the absence of migration, as occurs in our experimental system[19] (see Figs 2 and 3). Note that host and parasite populations were founded with 10^6 individuals, and measures of local adaptation are calculated from all individuals. The figure illustrates the fact that, when measured at the scale of a single population, antagonistic coevolution yields large fluctuations of local adaptation through time[3,8,25]. However, when measured at the scale of the metapopulation (that is, when local adaptation is averaged over different populations), the effect of the migration treatment is more apparent. Phage migration increased phage local adaptation, whereas bacterial migration had little effect. Supplementary Fig. S2 gives means and standard deviations obtained after 1,000 runs for a range of parameter values.

To test the role of host and parasite migration on local adaptation experimentally, we first established six replicate populations of isogenic bacteria and phages in experimental microcosms (static glass tubes containing nutrient-rich medium). A 1% portion of culture was transferred (an average of about 2×10^7 bacteria and 10^4 phages) to a fresh microcosm every 2 days, for a total of 73 transfers (about 500 bacterial generations) before experimental treatments, to permit between-population genetic divergence[19]. Each of these diverged cultures were used to seed five new cultures (a total of 30 cultures), each of which was exposed to one of five treatments: first, a no-migration control; second, 10% bacteria migration, 0% phage migration; third, 0.1% bacteria migration, 0% phage migration; fourth, 0% bacteria migration, 10% phage migration; and last, 0% bacteria migration, 0.1% phage migration. Cultures were propagated for a further 12 transfers, but bacteria and phages were isolated from each other by chemical means before transfer to a fresh microcosm. In the migration treatments, the specified amount of culture of the migrated species was taken from each replicate within a treatment, added to a common pool and mixed; the same volume was then added back to the replicates. Each migrated replicate was used to inoculate a fresh microcosm along with the previously isolated unmigrated species from the same replicate. If no migration was meant to occur (control populations), isolated bacteria or phages were simply inoculated back together into fresh microcosms. Culture samples were frozen every second transfer. Parasite local adaptation (as defined above) was assayed before the migration treatments (transfer zero), and at six subsequent times (every second transfer). This assay involved measuring the sensitivity of 20 independent bacterial colonies from each population to both sympatric phage populations, and the five allopatric phage populations within the same treatment.

Our experimental data were consistent with our simulations (compare Figs 1 and 2). First, as with other recent experimental data[20], we found local adaptation to vary considerably through space and time. Second, phage migration regimes rapidly changed patterns of local adaptation, and resulted in an increase in phage local adaptation, whereas bacterial migration seemed to have little effect. To analyse these data, we calculated mean local adaptation through time (transfers 2, 4, 6, 8, 10 and 12) for each population, and then fitted migration treatment as a five-level factor and local adaptation of starting population (at transfer zero) as a covariate in a General Linear Model. This analysis revealed significant differences in mean phage local adaptation between treatments (Fig. 3; $F_{4,24} = 3.30$, $P = 0.027$), as well as an almost significant effect ($P = 0.06$) of starting local adaptation. Simplification of the statistical model by pooling treatments[26] revealed no significant difference between the parasite migration treatments, and no differences between the host migration and the no-migration treatments (Fig. 3; $F_{3,24} = 0.08$, $P > 0.5$). Thus parasite migration, regardless of the rate, increased parasite local adaptation relative to when either host migration or no migration occurred ($F_{1,27} = 14.40$, $P = 0.001$).

It is interesting that the effect of migration was asymmetric: parasite migration increased parasite local adaptation relative to unmigrated populations, but host migration did not decrease parasite adaptation. This is likely to be because parasites are locally maladapted in the absence of any migration, and host migration is unable to increase parasite local maladaptation any further. Such a result was obtained in our theoretical analysis when the strength of selection on the host was higher than on the parasite ($\sigma_h > \sigma_p$): the parasite is expected to be locally maladapted when there is no migration, and the benefits of increased genetic variation resulting from host migration are diluted by stronger selection (Supplementary Fig. S2). An alternative explanation for this asymmetry is that within-population genetic variation for bacterial resistance may be greater than within-population genetic variation for phage infectivity; hence phages are locally maladapted in the absence of migration, and increased bacterial migration provides relatively little additional genetic variation for resistance traits. A difference in within-population genetic variation could result from the larger genome (hence more ways in which to generate resistance)[27] and population sizes[19] of bacteria than those of phages.

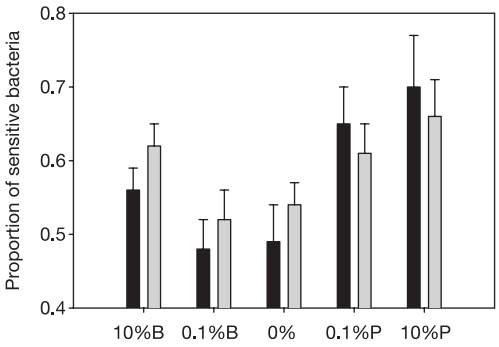

Figure 4 | Mean parasite infectivity. Shown are the proportions of sensitive bacteria to sympatric (black bars) and allopatric (grey bars) phage populations (averaged over six populations and six time points, namely T_2, T_4, T_6, T_8, T_{10} and T_{12}) for different migration treatments. Results are means ± s.e.m. across populations. P, phage migration; B, bacterial migration.

We also determined how host and parasite migration affected host local adaptation: the difference in performance between local and foreign hosts. Although the sign-independent mean values of host local adaptation were the same as parasite local adaptation, the variance was much greater, resulting in no significant effect of migration treatment ($F_{4,24} = 1.02$, $P = 0.4$). How can we explain this discrepancy? In this system, bacterial resistance genes that are beneficial against any particular phage populations (local resistance) are likely to be beneficial against all phage populations (global resistance; there is a strong covariance between local and global resistance; see Supplementary Information). Some bacterial populations will therefore be more resistant than other bacterial populations to all phage populations, hence obscuring evidence of host local adaptation. By contrast, the covariance between local and global infectivity of phage was much weaker, favouring the detection of parasite local adaptation.

An increased probability that either host or parasite will be locally adapted through differential migration is believed to result from host or parasite having an evolutionary advantage, and hence being ahead in a coevolutionary arms race[8–11]. We therefore expected average levels of phage infectivity to sympatric bacteria (defined as 'local performance'; see Supplementary Information) to be higher when phages were migrated than when there was no migration or bacterial migration. Our analysis, which also includes sympatric infectivity before migration treatments as a covariate, support this prediction (Fig. 4; $F_{4,24} = 5.61$, $P = 0.002$). Differences in sympatric infectivity between treatments may reflect two, not mutually exclusive, beneficial effects of migration: first, the introduction of genes that are beneficial for infecting a specific host population (the significant increase in phage local adaptation resulting from phage migration demonstrates that this process occurred); and second, the introduction of genes that are useful for phage infectivity across all populations[28] (this possibility was not considered in our theoretical analysis). To investigate the importance of the latter process, we determined how migration rates affected average infectivity on allopatric bacteria, also fitting allopatric infectivity before migration as a covariate. We found a significant effect of migration treatment (Fig. 4; $F_{4,24} = 3.06$, $P = 0.04$), indicating that migration might also allow universally beneficial genes to spread through populations. Note that starting sympatric and allopatric infectivity (at transfer zero) were significant positive predictors of sympatric and alloptaric infectivity, respectively, in the above analyses ($P < 0.01$), emphasizing the significance of historical contingency in current infectivity patterns.

We have thus demonstrated experimentally that parasite migration can provide an evolutionary advantage to parasites in a host–parasite coevolutionary arms race, and can therefore result in parasite local adaptation. That migration can enhance local adaptation is somewhat counterintuitive, given that migration can homogenize populations. However, our data are consistent with theory, which indicates that low rates of migration might increase within-population genetic variation of the migrated species, increasing evolutionary potential. We have yet to address whether local adaptation is influenced by relative migration rates when both species migrate, as predicted by theory[8–11]. The global scale of human interaction indicates that these results might have important implications for the evolution of human diseases. Parasites will inevitably accompany humans, but parasites will probably remain in the locality and contribute to the local gene pool more often than their hosts. Global interactions may therefore not only increase parasite transmission but may also increase the infectivity of parasites to their local host populations.

METHODS

Generation of divergent populations. Six replicate populations were initiated with approximately 10^8 cells of isogenic *Pseudomonas fluorescens* SBW25 (ref. 29) and about 10^5 particles of phage SBW25ϕ2 (ref. 19). Cultures were grown at 28 °C in 6 ml of King's media B (KB) in static 30-ml glass universal flasks with loose plastic lids. Every 48 h, 60 μl of culture was transferred to a fresh microcosm for a total of 73 transfers, to allow divergence between populations.

Isolation of bacteria and phages. The experiments required bacteria and phage to be isolated from each other. To isolate phage from bacteria, 100 μl of chloroform was added to 900 μl of culture, vortex-mixed and then centrifuged at 13,000 r.p.m. for 2 min (ref. 19). This lysed and pelleted the bacterial cells to the bottom of the tubes, leaving a suspension of phages in the supernatant (chloroform is denser than the supernatant and sits at the base of the tube). These were stored at 4 °C until needed. To isolate bacteria, a 5% solution of Virkon (a commercially available disinfectant) in water was made. This was added to KB to a concentration of 0.37% Virkon. A 60-μl sample of culture was added to 6 ml of the Virkon/KB solution in glass universal flasks, and left for 24 h, static at 28 °C. This procedure left the bacteria viable and completely phage free. A 60-μl portion of the Virkon cultures was added to a fresh static glass universal flask containing 6 ml of KB, and grown for one day at 28 °C to give a phage-free and Virkon-free stock. Phage-free bacterial cultures were stored in 20% glycerol solution at −80 °C. The potential presence of phages in Virkon-treated cultures was regularly tested for by plating out the cultures on to semisolid agar seeded with *Pseudomonas fluorescens* strain SBW25 and incubated at 28 °C for 24 hr. Plaques would have indicated the presence of phages; in all cases the test proved negative.

Migration regimes. Five treatments were used for this experiment: control; 10% bacteria migration, 0% phage migration; 0.1% bacteria migration, 0% phage migration; 0% bacteria migration, 10% phage migration; and 0% bacteria migration, 0.1% phage migration. A 60-μl sample of culture from the six diverged cultures was inoculated into five new microcosms per diverged culture (T_0), one for each of the treatments (six replicates per treatment, five treatments, yielding a total of 30 microcosms), and cultured for one transfer (48 h; T_1), after which bacteria or phages were isolated from each culture if they were not to be migrated (as described in the previous section). When phages or bacteria were migrated, 10% or 0.1% (depending on the migration regime) of culture from each of the six populations within a treatment was removed to a common pool, and mixed. The same volume of culture removed from the replicates was removed from the common pool and added back to the replicates. The organism to be migrated was isolated from the culture (as detailed in the previous section) and the rest of the culture was discarded. For each replicate, 60 μl of the isolated, migrated organism was added to a fresh microcosm along with 60 μl of the previously isolated unmigrated organism. In unmigrated control replicates, bacteria and phages were isolated from each other at each transfer, using the same chemical treatments as the migrated replicates, then 60 μl of the isolated bacteria and phages were used to inoculate fresh microcosms. Cultures were frozen every two transfers at −80 °C in 20% glycerol. Phages were isolated every two transfers and stored for local adaptation assays. Populations were evolved for 12 experimental transfers.

Local adaptation assay. We measured local adaptation in terms of bacterial resistance and phage infectivity. Every possible bacteria–phage pairwise interaction within each treatment was measured (36 (that is, 6 × 6) per treatment). For each interaction, 20 independent bacterial colonies were streaked across a 20 μl line of phages that had been dried on a KB agar plate[19]. Resistance and infectivity were measured as a binary trait, where a bacterial colony was classed as

sensitive if there was any visible inhibition of growth when exposed to the phage, and resistant otherwise. These assays were performed after 2, 4, 6, 8, 10 and 12 transfers, as well as immediately before the migration treatments.

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