

Terminal investment induced by a bacteriophage in a rhizosphere bacterium [v1; ref status: awaiting peer review, <http://f1000.es/SZsivDP>]

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v1 First published: 02 October 2012, 1:21 (doi: 10.3410/f1000research.1-21.v1)

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How to cite this article: Poisot T, Bell T, Martinez E, Gougat-Barbera C, Hochberg ME (2012) Terminal investment induced by a bacteriophage in a rhizosphere bacterium [v1; ref status: awaiting peer review, <http://f1000.es/SZsivDP>] *F1000 Research* 1:21 (doi: 10.3410/f1000research.1-21.v1)

Abstract Despite knowledge about microbial responses to abiotic stress, few studies have investigated stress responses to antagonistic species, such as competitors, predators and pathogens. While it is often assumed that interacting populations of bacteria and phage will coevolve resistance and exploitation strategies, an alternative is that individual bacteria tolerate or evade phage predation through inducible responses to phage presence. Using the microbial model *Pseudomonas fluorescens* SBW25 and its lytic DNA phage SBW25 ϕ 2, we demonstrate the existence of an inducible response in the form of a transient increase in population growth rate, and found that the response was induced by phage binding. This response was accompanied by a decrease in bacterial cell size, which we propose to be an associated cost. We discuss these results in the context of bacterial ecology and phage-bacteria co-evolution.

Introduction

Pathogens are ubiquitous in natural communities¹ and the antagonistic interactions they establish with their hosts are recognized as one of the main drivers of evolutionary diversification^{2,3}. Hosts can reduce the impact of pathogens through three non-mutually exclusive processes⁴: (i) avoidance of either infected individuals, habitats where the pathogen is prevalent, or of the pathogen itself⁵, (ii) resistance to the actual infection process or post-infection immune defences⁶, and (iii) tolerance⁷. Research on these responses has generally focused on animal and plant models, but there is growing appreciation that microbes, particularly bacteria, can exhibit similar responses. For instance, bacteria can be selected for heightened levels of genetic resistance towards infection by pathogens^{8–10}. On the other hand, although bacteria are

known to display plastic responses to various types of environmental stresses^{11,12} and to competition¹³, it is unknown whether they can do so when faced with natural enemies such as bacteriophages.

Plastic responses are an adaptive phenotypic change following an environmental stimulus, occurring without a concurrent change in the genotype¹⁴. They may involve behavioural, physiological or phenological changes^{15,16}, and be triggered by direct or indirect contact with the stimulus¹⁷ or through communication with neighbouring organisms¹⁸. Phenotypic plasticity is considered to be a genetic adaptation to variable environments, but given the diversity of associated mechanisms and behaviours, it is not known to what extent different stimuli translate into different responses^{15,19}.

Individual-level interactions between bacteria and phage may be conducive to induced responses. The first step of bacteriophage infection is the binding of phage proteins to bacterial surface proteins²⁰, which then triggers conformational changes to both proteins²¹. Surface proteins used by the bacterium for signal transduction are known to be targets of bacteriophage adsorption²² and as such could trigger a response when bacteriophage binding is detected. Such a response would allow a bacterium to react to the pathogen and to eventually either evade or reduce the effects of the infection. Lytic phages are prime candidates for organisms against which bacteria may have evolved a stress response, because they typically interact with their host over short timescales, and death is inevitable once the phage has injected its DNA into a sensitive bacterial cell.

In addition, bacteriophages are widely distributed in the environment²⁰ and interact with their hosts over relatively small spatial scales²³ and throughout most of the year^{24,25}. This could select for the expression of induced structural, physiological or behavioural responses to different enemies. Also, bacteria employ signalling pathways and have a known ability to communicate within populations²⁶. Such pathways could induce and synchronise inducible responses before predators and pathogens are encountered, or at least before they have spread through the population, or before the point beyond which cell death is certain. All of these factors suggest that plastic stress responses to phage should be a common feature of bacterial cells and that such responses would have important repercussions for ecological and evolutionary interactions between phage and bacterial populations. Although molecular responses of bacteria to bacteriophages have been characterized²⁷, the behavioral, ecological, and selective consequences of such responses are not known.

Here we demonstrate that when confronted with phage, bacteria express transient increases in division rate at a cost to individual biomass accumulation²⁸. Specifically, we employ the rhizosphere bacterium *Pseudomonas fluorescens* SBW25²⁹ to investigate how its population growth rate is affected by exposure to inactivated populations of its lytic bacteriophage SBW25Φ2³⁰. We find that bacteria exposed to inactivated phage increase their fission rate nearly two-fold at 24 hours post-exposure. This is followed by a continual decrease in fission rate relative to the control. We also show that bacteria exposed to inactivated phage were smaller in size compared to controls. All of these effects were enhanced as the density of inactivated phage was increased. The results are consistent with a behavioural strategy that increases allocation to reproduction under stressful conditions (i.e., “terminal investment”). Terminal investment is well characterised for other host-parasite associations³¹, but to our knowledge has not previously been observed in bacteria and phage.

Results

Bacteria exposed to UV-inactivated phage display a statistically significant higher growth rate over the first 24 hours post-exposure than non-phage controls (Kruskal-Wallis, $df=3$, $P = 0.006$; Fig 1). After this period, the estimated doubling time of exposed bacteria increased (i.e., their populations grew slower), and did so for the next 48 hours. This decrease in growth rate compared to controls is suggestive of a cost to the higher fission rate observed over the first 24 hours (Fig 1). During the fourth day post-exposure, control and treatment bacteria showed no significant differences in doubling time (KW, $df=3$, $P > 0.05$). That exposed bacteria returned to their ancestral growth rate suggests that the response over the first 24 hours was due to phenotypic plasticity and not selection on faster growing genotypes. There was a marginally significant effect on population growth for bacteria exposed to different phage concentrations (KW, $df=2$, $P < 0.02$), suggesting that the encounter rate between bacteria and phage is important in determining the population-level strength of the fission response.

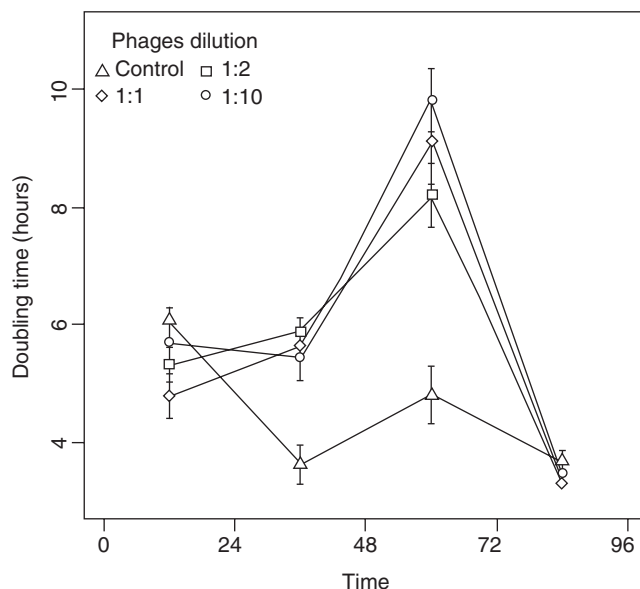


Figure 1 Maximum doubling time (in hours) of biomass produced by bacteria exposed to different concentrations of UV-inactivated phage. This was measured for four consecutive days following four hours exposure. Bacteria exposed to phage grew significantly faster than controls over the first day, and then expressed an apparent cost in terms of smaller cell size that attenuated by the fourth day. Central points are the means of 12 replicates, and the bars are standard errors.

Dataset 1: Bacterial doubling time, expressed in hours, as a function of the treatment

<http://dx.doi.org/10.6084/m9.figshare.95948>

We hypothesized that faster doubling times would come at a cost to cell size, since cells would have less time to metabolize and convert absorbed nutrients into cell structure. Twenty-four hours post-exposure, we found that phage-treated bacteria were two to three times smaller (as measured by mean cellular width) than the control (KW, $df=3$, $P < 0.0001$; Fig 2). This difference in size gradually decreased over the following 3 days, but in contrast to growth rate (Fig 1), bacteria did not attain their ancestral cell size by the end of the experiment (Fig 2). Analyses of the distribution of several flow cytometry profiles showed that a difference in cell shape is unlikely to explain this result (see data associated to this article). Finally, observations using a transmission electron microscope showed that the cells remained rod-shaped for all treatments.

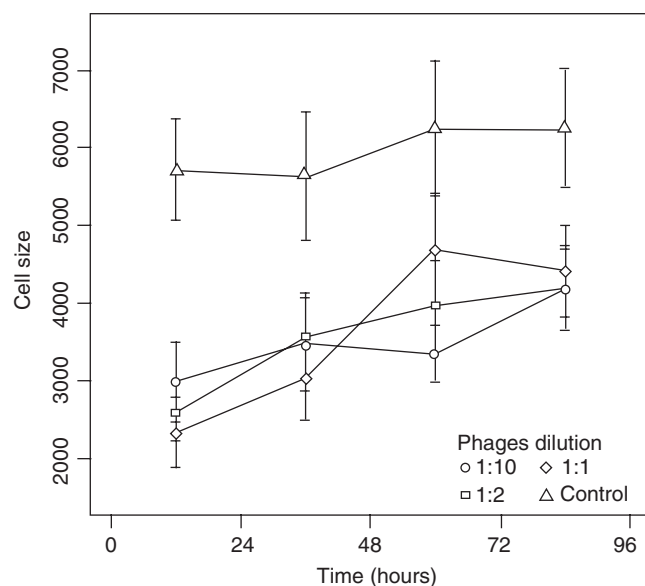


Figure 2 Mean bacterial cell size (forward scatter parameter) exposed to different concentrations of UV-inactivated phage, as per the method in Figure 1. Bacteria exposed to phage at different concentrations do not significantly differ in size. Points and bars are the same as in Figure 1.

Dataset 2: Raw flow cytometry data

<http://dx.doi.org/10.6084/m9.figshare.95949>

We did not observe any difference in the impact of live phage on bacterial populations exposed to the different treatments (KW, $df=2$, $P = 0.153$), suggesting that the inducible response does not alter bacteria resistance to phage predation.

Discussion

Our experiments reveal a previously unexplored behavioural response to bacteriophage predation: phage induce bacteria to

reproduce earlier in their cell cycle. We hypothesize that this response increases the survival chances of progeny under natural conditions and demonstrate that this behaviour comes at a fitness cost of reduced size of daughter cells. Our experiments with UV-inactivated phage further demonstrate that this response is specifically due to phage binding.

These results support and extend both theoretical³² and empirical^{33,34} predictions that victims may lessen the fitness impact of their natural enemies through early reproduction, to cases where phenotypic responses are plastic and temporary. Increased allocation to reproduction in stressful environments—termed “fecundity compensation” or “terminal investment”³¹—although never studied in bacteria–phage associations to our knowledge—has been extensively studied for other host–parasite (or organism – stressor) interactions. Terminal investment is characterized by increased reproductive rate or the earlier onset of reproduction, if the prospect of future reproduction is low³⁵. Examples of such responses include faster host maturation³⁶, increased oviposition rate³⁷, and the modification of traits involved in the onset of reproduction^{38,39}.

Phenotypically plastic responses are important in that they allow individuals to cope with environmental change during their lifetimes⁴⁰. As such, plasticity is expected to be favoured in variable environments when the costs of induction and phenotypic change compensate for probabilistic (expected) fitness loss⁴¹. Although it is difficult to generalize about constitutive costs of resistance across biological systems^{42,43}, limited evidence suggests that genetically evolved, constitutive resistance in bacteria to their lytic phage could have costs of as much as 5–10% to relative fitness⁴⁴.

We employed inactivated bacteriophages to evaluate how phage contact with the bacterial outer membrane mediates bacterial responses. Bacteria could be selected to exhibit an escape response in several, non-mutually exclusive ways. First, non-virulent phage may signal the presence of virulent phage in the local environment (i.e., the bacterium does not perish following initial phage contact). Senescent (inactive) phage are present in natural environments²⁵, and many phages bind to outer membrane proteins without being infective (e.g. the bacterium is resistant;⁴⁴). Moreover, it is possible that phage could detach if they sense the host to be unsuitable⁴⁵. Second, when phage infect the bacterium there may be a ‘race’ between the time it takes a bacterial cell to divide (and potentially survive) and the point of no recovery associated with the maturation of phage progeny and bacterial cell lysis. Third, the response may be a consequence of lysogens competing with lytic phages for host exploitation; the latter could benefit from early host reproduction in the presence of lytic competitors. However, sequencing of the *P. fluorescens* SBW25 genome revealed a low abundance of prophage-like regions⁴⁶.

We were not able to determine whether the bacteria or the phage benefit from faster bacterial reproduction, and the literature reports effects both of facilitation and decrease in host metabolism upon infection⁴⁷. Previous theoretical work suggests that phage productivity increases in bacteria with short life-cycles⁴⁸. This is supported by recent empirical study employing the same strain of *P. fluorescens*⁴⁹. Assuming that the physiological mechanisms involved in fission rate increases are the same in the two experiments, this suggests that rapid multiplication is not adaptive for the bacterium. Upon exposure to phage, bacteria reproduce faster, but experience a persistent reduction in individual size. Smaller cells have less surface area, and assuming that the density of receptor proteins does not change with cell size, this suggests that they will have lower encounter rates with phage. One possibility is that cell division allows bacterial cells to concentrate phage in one of the daughter cells^{50,51}, resulting in some progeny managing to escape the pathogen. Future studies should therefore focus on the possible adaptive nature of this response for both bacterium and phage.

Methods

Bacteria cultures

Ancestral *Pseudomonas fluorescens* SBW25²⁹ were inoculated into 30 ml microcosms containing 6 mL of King's B medium (KB), and allowed to grow under alternating rotational agitation (200 rpm for 1 minute every 30 minutes). Every 48 h following plating on solid agar, 10 CFU of the smooth morphotype were transferred into fresh KB medium. After 10 transfers, the culture was composed of smooth morphotypes only. We continued this selection procedure for another 10 transfers and then arbitrarily isolated a single CFU, which was used for all experiments described below. Experiments were conducted at 28°C in KB medium under constant rotational agitation (200 rpm).

Phage cultures

We grew an arbitrarily selected clone of the ancestral phage SBW25Φ2³⁰ on an exponentially growing culture of fixed smooth *P. fluorescens* SBW25 in 3 mL of KB for 48 hours. This resulted in a culture containing approximately 10⁸ phage per ml. The sample was then centrifuged for 3 minutes at 8000 rpm in a 1.5 ml Eppendorf tube, and the pellet discarded. Centrifugation was repeated three times to ensure all bacteria were removed (see Suppl. Fig. 1). Phages were then isolated by centrifuging the remaining supernatant for 8 minutes at 13000 rpm, and inoculating the pellet into fresh KB medium. The sample was thoroughly vortexed and exposed to UV light (Model 4.LC, Vilber Lourmat, Deutschland, 254 nm wavelength) at 5 cm distance for 4 hours. Extensive pilot studies demonstrated that this method was sufficient to kill all phage (see Suppl. Fig. 2).

Preliminary tests

We conducted a series of preliminary tests to verify how UV-inactivated phage affected bacterial hosts. First, observations under a transmission electron microscope showed that UV-inactivated phage were still intact and able to bind to their bacterial hosts. Second, we checked that bound UV-inactivated phage did not introduce phage DNA into the bacteria. This was done by inoculating 1 ml of UV-inactivated phage into 6 overnight bacterial cultures. Inactivated phage were allowed 4h to attach to the bacterial outer membrane. We separated phage and bacterial fractions by filtration using a 0.2 µm filter. We then conducted a full DNA extraction (WholeBlood NucleoSpin DNA extraction kit, Macherey-Nagel) of the filter. PCR was done using *TPV1f* (GATGTGAGAAAGCGATACACGG) and *TPV1r* (GAGAGAAGCGGGAGAGTGAA) sequences developed for this study, which selectively amplify a 550 bp fragment of the phage DNA and a 1200 bp fragment of the bacterial DNA (see Suppl. Fig. 1 for detailed protocols). We did not find any evidence that UV-inactivated phage was present in samples putatively containing bacteria only, thus confirming that the DNA of inactivated phage was not incorporated in the bacterial cell.

Experiments using UV-inactivated phage

We conducted an experiment to understand how UV-inactivated phage affected bacterial behaviour. Fixed smooth SBW25 bacteria were first cultivated in 6 ml KB in 30 mL universal glass vials. 20 µL of exponentially growing bacteria (*c* 10⁴ bacterial cells) were transferred into fresh KB medium with either no phage or UV-inactivated phage at ratios of 1:10, 1:2, and 1:1 (corresponding to approximately 10⁶, 5x10⁶, and 10⁷ phage per ml), and then allowed to interact for 4 hours under alternating shaking (200 rpm for 1 minute every 30 minutes). Bacteria were then separated from bound phage by centrifuging (see above) and placed in fresh KB medium. 1% of each population was transferred every 24 hours into new KB medium. Each of the 4 treatments was replicated 6 times and arranged arbitrarily in a rack for incubation.

Measures

Biomass doubling time (used as a proxy for population fitness) was measured in a Fluostar Optima spectrophotometer (28°C, constant agitation, 250 measures at 650 nm over 24 hours) each day, using the following formula:

$$(1) \quad D_t = [\Delta t \ln(2)] / [\ln(N^*) - \ln(N_0)]$$

where N^* and N_0 are the total biomasses (measured as optical density, OD) before and after the exponential growth phase, and Δt is the duration of the exponential phase. Exponential phase was determined by conducting a series of windowed

linear regressions over the full growth curve, and retaining the part of the curve with the largest slope (computer code given in suppl. materials part 3).

Individual cell size was measured by flow-cytometry using a FacsCantoII (BD BioSciences, San Jose, California, USA), and data (forward scatter) were analysed using the *flowCore* package⁵² in R 2.12.0⁵³. Each measure was performed on a sample of 2×10^5 cells without dyes.

We also estimated the sensitivity of the different treatments to live phage by measuring changes in bacterial populations. At each 24-hour transfer, 1% of the bacterial population was placed in 2 mL of fresh KB, and 20 μ L of amplified phage (*ca* 10^8 viral particles) were added (a control without phage was conducted simultaneously). Bacteria CFUs were counted on solid agar after 48 hours of incubation to estimate population size.

Due to non-normality of the data as assessed by a Shapiro test, we used a Kruskal-Wallis test to determine the significance of the between-treatments effects.

Authors contributions

TP, TB and MEH designed the research, TP and EM conducted the microbiology experiments, TP and CGB conducted the molecular biology experiments, TP, TB and MEH analyzed the results and wrote the paper, all authors contributed to revisions.

Competing Interests

No competing interests were disclosed.

Grant Information

TP is funded by a CNRS-Région Languedoc Roussillon doctoral grant. TB was funded by NERC. MEH is funded by ANR EvolStress (ANR-09-BLAN-099-01), by the Ec2Co "Cytrix" program, and the McDonnell Foundation (JSMF 220020294/SCS-Research Award).

Acknowledgments

We are indebted to Cédric Mongellaz for his assistance with flow cytometry, and the Montpellier RIO Imaging center for providing technical support. We thank Michael Brockhurst and Angus Buckling for discussions.

Supplementary material and figures

1. Molecular biology protocol and results

PCR cycle – 6 minutes at 95°C, then 30 cycles of 1 minute at 94°C, 1 minute at 55°C, 2 minutes at 72°C, then 10 minutes at 72°C

PCR buffer – 5 μ L of buffer, 4 μ L of primers at 10 pM/mL (for both TPV1f and TPV1r), 2 μ L of dNTP at 5pM/mL, 1.5 μ L of

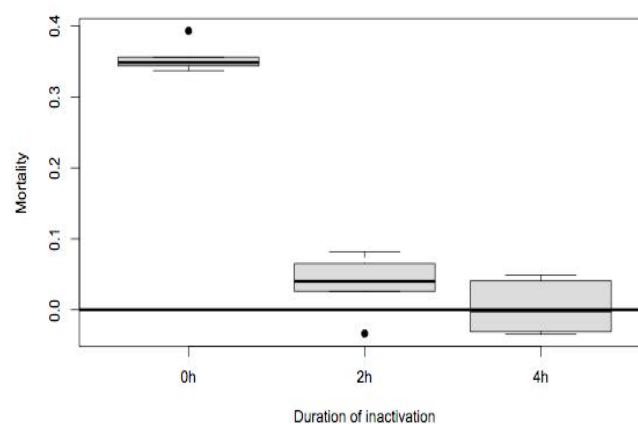
MgCl₂ at 25mM, 5.35 μ L of H₂O, 3 μ L of sample DNA, 0.15 μ L of TaqPol - conducted with a GoTaq FlexiDNA Polymerase M8301 kit from Promega.



Suppl. figure 1 - Sample gel obtained on 9 total DNA extractions (A-C: bacteria and phage, D-F: phage only, following extraction as explained in text, G-I: bacteria following exposure to inactivated phage, whose DNA was extracted after removal of inactivated phages). The primers TPV1f and TPV1r yield a 1200 bp amplicon in the bacteria, and a 500 bp amplicon in phages. Our separation method for bacteria and phage was complete, since only DNA of the intended organism was found in any given sample.

2. Preliminary experiments and phage

We verified the efficiency of the phage inactivation protocol by incubating the bacterial strain used for the main experiment with either live phage or phage exposed to UV for 2hrs or 4hrs. We measured the Malthusian fitness of 6 host populations near carrying capacity at low temperature (4°C, growth restrictive) over the course of 24hrs (the difference with the experiment presented in the main text is that inactivated phage *were not removed* over the course of this pilot study).



Suppl. figure 2 - Bacterial mortality as a function of phage inactivation. Because bacteria do not grow at 4°C, we can directly measure phage-induced mortality. After 4 hours of exposure to UV, we observed that phages do not introduce significant mortality in the bacterial population. Kruskal-Wallis test (df = 2, p = 0.02) reveals differences between treatments, with 0h and 2h being significantly different (t-test, df = 7, p < 10^{-5}), 0h / 4h being significantly different (t-test, df = 8, p < 10^{-5}), and 2h and 4h being similar (t-test, p = 0.16) - all p-values were Bonferroni-corrected to account for multiple testing. Similarly, 2h and 4h are not significantly different from 0 (p-values of 0.14 and 0.89 respectively, after correction).

3. Determination of the maximal growth rate (R code)

```
givegrowth = function (y, x = c(1:length(y)), bw = 12)
## y : optical density
## x : times of the measures
## bw : number of points to include in regression
{
  list.of.coeff <- NULL
  for (i in 1:(length(x) - bw)) {
    part.x <- x[i:(i + bw)]
```

```
    part.y <- y[i:(i + bw)]
    cur.lm <- lm(part.y ~ part.x)$coeff[2]
    list.of.coeff[i] <- cur.lm
  }
  result <- -max(list.of.coeff)
  pos <- -match(max(list.of.coeff), list.of.coeff)
  coeff <- -lm(y[pos:(pos + bw)] ~ x[pos:(pos +
bw)])$coeff
  return(as.numeric(coeff[2]))
}
```

References

- Lafferty, K.D., Dobson, A.P. & Kuris, A.M. (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences*, **103**, 11211–6.
- Coberly, L.C., Wei, W., Sampson, K.Y., Millstein, J., Wichman, H.A. & Krone, S.M. (2009) Space, time, and host evolution facilitate coexistence of competing bacteriophages: theory and experiment. *The American Naturalist*, **173**, E121–38.
- Weitz, J.S., Hartman, H. & Levin, S.A. (2005) Coevolutionary arms races between bacteria and bacteriophage. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 9535–40.
- Boots, M. & Bowers, R.G. (1999) Three Mechanisms of Host Resistance to Microparasites — Avoidance, Recovery and Tolerance — Show Different Evolutionary Dynamics. *Journal of Theoretical Biology*, **1**, 13–23.
- Hart, B.L. (1990) Behavioral adaptations to pathogens and parasites: five strategies. *Neuroscience & Biobehavioral Reviews*, **14**, 273–294.
- Ebisuzaki, K. & Jellie, S.B. (1981) Postinfection control in T4 bacteriophage infection: inhibition of the rep function. *Journal of Virology*, **37**, 893.
- Miller, M.R., White, A. & Boots, M. (2005) The evolution of host resistance: tolerance and control as distinct strategies. *Journal of Theoretical Biology*, **236**, 198–207.
- Forde, S.E., Beardmore, R.E., Gudelj, I., Arkin, S.S., Thompson, J.N. & Hurst, L.D. (2008) Understanding the limits to generalizability of experimental evolutionary models. *Nature*, **455**, 220–223.
- Gruner, D.S., Kolekar, A., McLaughlin, J.P. & Strong, D.R. (2009) Host resistance reverses the outcome of competition between microparasites. *Ecology*, **90**, 1721–1728.
- Poullain, V., Gandon, S., Brockhurst, M.A., Buckling, A. & Hochberg, M.E. (2008) The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. *Evolution*, **62**, 1–11.
- Storz, G.T. & Hengge-Aronis, R. (2000) *Bacterial Stress Responses*. ASM Press.
- Wang, S., Deng, K., Zaremba, S., Deng, X., Lin, C., Wang, Q., Tortorello, M.L. & Zhang, W. (2009) Transcriptomic Response of *Escherichia coli* O157: H7 to Oxidative Stress. *Applied & Environmental Microbiology*, **75**, 6110.
- Kümmerli, R., Jiricny, N., Clarke, L.S., West, S.A. & Griffin, A.S. (2009) Phenotypic plasticity of a cooperative behaviour in bacteria. *Journal of Evolutionary Biology*, **22**, 589–98.
- Schlichting, C.D. & Piglucci, M. (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates Sunderland, MA.
- Frank, S.A. (1993) A model of inducible defense. *Evolution*, **47**, 325–327.
- Schlichting, C.D. (1986) The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, **17**, 667–693.
- Maleck, K. & Dietrich, R.A. (1999) Defense on multiple fronts: how do plants cope with divers enemies? *Trends in Plant Science*, **4**, 215–219.
- Shapiro, J.A. (2007) Bacteria are small but not stupid: cognition, natural genetic engineering and socio-bacteriology. *Stud Hist Philos Biol Biomed Sci*, **38**, 807–819.
- Stanton, M.L., Roy, B.A. & Thiede, D.A. (2000) Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution*, **54**, 93–111.
- Abedon, S.T. (2008) *Bacteriophage Ecology: Population Growth, Evolution, and Impact of Bacterial Viruses*. Cambridge University Press.
- Ossiboff, R.J., Zhou, Y., Lightfoot, P.J., Prasad, B. & Parker, J.S.L. (2010) Conformational Changes in the Capsid of a Calicivirus upon Interaction with its Functional Receptor. *Journal of Virology*, **84**, 5550.
- Menge, D.N.L. & Weitz, J.S. (2009) Dangerous nutrients: evolution of phytoplankton resource uptake subject to virus attack. *Journal of Theoretical Biology*, **257**, 104–115.
- Vos, M., Birkett, P.J., Birch, E., Griffiths, R.I. & Buckling, A. (2009) Local Adaptation of Bacteriophages to Their Bacterial Hosts in Soil. *Science*, **325**, 833+.
- Asheford, K.E., Norris, S.J., Fry, J.C., Bailey, M.J. & Day, M.J. (2000) Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. *Applied & Environmental Microbiology*, **66**, 4193–4199.
- Maurice, C.F., Bouvier, T., Comte, J., Guillemette, F. & Del Giorgio, P.A. (2010) Seasonal variations of phage life strategies and bacterial physiological states in three northern temperate lakes. *Environmental Microbiology*, **12**, 628–41.
- Waters, C.M. & Bassler, B.L. (2005) Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.*, **21**, 319–346.
- Huvel, M., Toni, T., Sheng, X., Thorne, T., Jovanovic, G., Engl, C., Buck, M., Pinney, J.W. & Stumpf, M.P.H. (2011) The Evolution of the Phage Shock Protein Response System: Interplay between Protein Function, Genomic Organization, and System Function. *Molecular Biology and Evolution*, **28**, 1141–55.
- St-Pierre, F. & Endy, D. (2008) Determination of cell fate selection during phage lambda infection. *Proceedings of the National Academy of Sciences*, **105**, 20705.
- Rainey, P.B. & Travisano, M. (1998) Adaptive radiation in a heterogeneous environment. *Nature*, **394**, 69–72.
- Buckling, A. & Rainey, P.B. (2002) The role of parasites in sympatric and allopatric host diversification. *Nature*, **420**, 496–499.
- Clutton-Brock, T.H. (1984) Reproductive Effort and Terminal Investment in Iteroparous Animals. *The American Naturalist*, **123**, 212.
- Hochberg, M.E., Michalakakis, Y. & De Meeûs, T. (1992) Parasitism as a constraint on the rate of life-history evolution. *Journal of Evolutionary Biology*, **5**, 491–504.
- Michalakakis, Y. & Hochberg, M.E. (1994) Parasitic effects on host life-history traits: a review of recent studies. *Parasite*, **1**, 291–294.
- Mitchell, S.E., Rogers, E.S., Little, T.J. & Read, A.F. (2005) Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, **59**, 70–80.

35. Minchella, D.J. (1985) Host life-history variation in response to parasitism. *Parasitology*, **90**, 205–216.
36. Lafferty, K.D. (1993) The marine snail, *Cerithidea californica*, matures at smaller sizes where parasitism is high. *Oikos*, **68**, 3–11.
37. Adamo, S.A. (1999) Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. *Animal Behaviour*, **57**, 117–124.
38. Blair, L. & Webster, J.P. (2007) Dose-dependent schistosome-induced mortality and morbidity risk elevates host reproductive effort. *Journal of Evolutionary Biology*, **20**, 54–61.
39. Chadwick, W. & Little, T.J. (2005) A parasite-mediated life-history shift in *Daphnia magna*. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 505–9.
40. Chevin, L.-M., Lande, R. & Mace, G.M. (2010) Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biology*, **8**, e1000357.
41. Meyers, L.A. & Bull, J.J. (2002) Fighting change with change: adaptive variation in an uncertain world. *Trends in Ecology & Evolution*, **17**, 551–557.
42. Coustau, C., Chevillon, C. & Ffrench-Constant, R. (2000) Resistance to xenobiotics and parasites: can we count the cost? *Trends in Ecology & Evolution*, **15**, 378–383.
43. Rigby, M.C., Hechinger, R.F. & Stevens, L. (2002) Why should parasite resistance be costly? *Trends in Parasitology*, **18**, 116–120.
44. Buckling, A., Wei, Y., Massey, R.C., Brockhurst, M.A. & Hochberg, M.E. (2006) Antagonistic coevolution with parasites increases the cost of host deleterious mutations. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 45–49.
45. Heineman, R.H., Springman, R. & Bull, J.J. (2008) Optimal foraging by bacteriophages through host avoidance. *The American Naturalist*, **171**, E149–57.
46. Sibley, M.W. et al. (2009) Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. *Genome Biology*, **10**, R51.
47. Nechaev, S. & Severinov, K. (2008) The elusive object of desire — Interactions of bacteriophages and their hosts. *Current Opinion in Microbiology*, **11**, 186–193.
48. Rabinovitch, A., Fishov, I., Hadas, H., Einav, M. & Zariitsky, A. (2002) Bacteriophage T4 Development in *Escherichia coli* is Growth Rate Dependent. *Journal of Theoretical Biology*, **216**, 1–4.
49. Escobar-Paramo, P., Faivre, N., Buckling, A., Gougat-Barbera, C. & Hochberg, M.E. (2009) Persistence of costly novel genes in the absence of positive selection. *Journal of Evolutionary Biology*, **22**, 536–543.
50. Ryter, A., Shuman, H. & Schwartz, M. (1975) Intergration of the receptor for bacteriophage lambda in the outer membrane of *Escherichia coli*: coupling with cell division. *Journal of Bacteriology*, **122**, 295.
51. Zeng, L., Skinner, S.O., Zong, C., Sippy, J., Feiss, M. & Golding, I. (2010) Decision Making at a Subcellular Level Determines the Outcome of Bacteriophage Infection. *Cell*, **141**, 682–691.
52. Lee, K., Hahne, F., Sarkar, D. & Gentleman, R. (2009) iFlow: A Graphical User Interface for Flow Cytometry Tools in Bioconductor. *Advances in Bioinformatics*, 103839.
53. R Development Core Team. (2008) R: A Language and Environment for Statistical Computing.