Chapter 20
Subtle Environmental Differences have Cascading Effects on the Ecology and Evolution of a Model Microbial Community*

Justin R. Meyer and Richard E. Lenski

Abstract Predicting ecological and evolutionary dynamics is challenging because the phenomena of interest emerge from complex nonlinear interactions between genomes, organisms, and environments. Complexity theory predicts that small changes in a basal element of an ecosystem can impact higher-order features such as population dynamics and biodiversity. Here we use a simple two-species laboratory system to demonstrate how slight alterations to the environment can have cascading effects on the ecology and evolution of that system. We cultured the bacterium *Escherichia coli* and a virus, phage λ, together in a carbon-limited medium. We varied the carbon source by supplying one of three similar sugars: glucose, maltose, or maltotriose. These sugars were chosen because we predicted they would impose varying degrees of constraint on the potential for the bacteria to evolve resistance to the phage. The sugars have different routes into the cell: both maltodextrins rely on the outer-membrane pore LamB, whereas glucose does not. LamB also serves as the receptor for λ attachment to the cell surface, and mutations that alter its structure or reduce its expression can confer resistance to λ. By varying the sugar and thereby the bacteria's reliance on LamB, we predicted they would evolve different types of resistance and engage in different coevolutionary trajectories with λ. We saw even more striking effects than expected. This simple resource manipulation caused differences in the bacteria's cost of resistance, which in turn affected population dynamics, community composition, coexistence, and coevolution. This cascade has important implications for predicting ecology and evolution. On the one

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* This paper was externally peer-reviewed.

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W. Banzhaf et al. (eds.), *Evolution in Action: Past, Present and Future*,
Genetic and Evolutionary Computation, https://doi.org/10.1007/978-3-030-39831-6_20

hand, it reveals that even subtle environmental differences can have large and complex effects, making predictions difficult. On the other hand, important features of the environment (here, the specific limiting resource) can sometimes be identified *a priori* given sufficient knowledge of the molecular biology and physiology of the organisms.

Key words: coevolution, experimental evolution, parasites, phage lambda, trade-offs

20.1 Introduction

Charles Darwin famously expressed the complexity of the living world in the closing paragraph of *The Origin of Species* [13]: "It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us." He envisioned that a handful of basic processes—reproduction, inheritance, variation, and the struggle for survival—could produce such complexity.

Today, the idea that interactions among a few simple processes can generate complex systems is the subject of complexity theory [31]. One implication of this theory is that subtle changes in the system can cause major transformations. If this sounds familiar, the idea was popularized in the movie Jurassic Park as the "butterfly effect." Applied to biology, one can imagine a scenario in which slight changes in the environment, say in average temperature, could impact the metabolism of organisms, alter the growth potential of populations, destabilize key predator-prey interactions, and cause ecosystem collapse. Here we report on a set of experiments that examine the idea that subtle changes to an environment can cause large changes in the ecology and evolution of interacting species.

Sensitivity to minor perturbations can occur in complex systems when interactions between components are strong, nonlinear, and produce feedbacks [31]. Viruses and their hosts offer a good example of such interactions, and they are pervasive throughout nature. They often have strong antagonistic effects, where the survival of one depends on the death or morbidity of the other. Their population dynamics are characterized by nonlinear functions [24], and their interactions readily generate feedbacks. One potential feedback is a coevolutionary arms race, in which the host evolves resistance and the virus evolves counter-defenses [24]. In line with complexity theory, viral-host coevolution is thought to contribute to many emergent properties of biological systems, including the production and maintenance of biodiversity [4, 6, 40], the evolution of more evolvable systems [30, 43], and the emergence of sexual reproduction [19]. Also consistent with complexity theory, co-

evolutionary trajectories are often highly sensitive to other environmental variables [18, 26].

Here we report results from experiments with a simple microbial community comprising just two species, a virus and its bacterial host. We manipulated the environment in a subtle way and documented how that manipulation altered the ecology and evolution of the community. The host is *Eschierichia coli* and the virus is a strictly lytic variant of phage λ. Previous studies have shown that *E. coli* and λ undergo an arms race in the laboratory [2, 5, 7, 21, 28, 32, 35, 41]. Resistance to λ typically evolves by mutations affecting the expression or amino-acid sequence of the receptor, LamB [5, 38]. *E. coli* expresses LamB to consume maltodextrins [9], which are chains of glucose molecules, such as maltose (two glucose units) and maltotriose (three units) (Fig. 20.1). By contrast, glucose usually enters the cell by another porin, OmpF, which wild-type λ cannot use. OmpF has a low affinity for maltose, and even lower for maltotriose [12]. We predicted that varying which of these three sugars we supplied would impose different constraints on the ability of *E. coli* to evolve resistance and thereby impact its coevolution with λ (see also [17, 24, 40]).

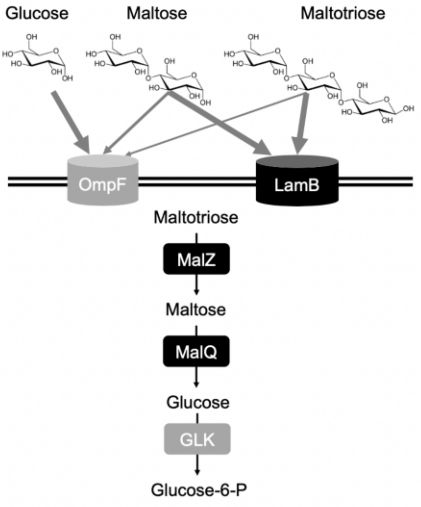


Fig. 20.1: Transport of the three sugars used in this study across the outer membrane (horizontal double line) via two different pore proteins and their initial metabolic conversions once in the cytoplasm. The thickness of the gray arrows represents schematically the relative importance of each pore type for each sugar's transport. The proteins shown in black are activated by MalT, whereas those shown in gray are not.

20.2 Materials and Methods

20.2.1 Strains

We used as starting hosts *E. coli* B strains REL606 and REL607 because their evolution in the laboratory has been well documented [3, 25, 37, 42]. Moreover, these strains readily coevolve with phage in a receptor-focused manner because they lack other defenses including restriction-modification systems, a functional CRISPR system, and the capacity to produce a broadly resistant mucoid phenotype [35, 36]. REL606 and REL607 differ by a mutation that prevents REL606 from growing on arabinose (Ara^-), which provides a marker that is selectively neutral [25, 39]. The λ strain that we used is cI26, an obligatorily lytic mutant provided by Donald Court (National Cancer Institute). This strain has a single nucleotide deletion that causes a frameshift in the *cI* repressor gene [28] that otherwise maintains lysogeny [20].

20.2.2 Experimental Procedures and Culture Conditions

Populations of phage λ and *E. coli* were cultured together in a minimal medium in which the growth of the *E. coli* population is limited by the available carbon. Three treatments were imposed by supplying one of three sugars: glucose, maltose, or maltotriose. For each community, we inoculated $\sim$100 phage and $\sim$1,000 cells in 10 mL of modified Davis Medium (DM) [25] with 10x the standard concentration of magnesium sulfate (1 μg mL^{-1}) and 125 μg mL^{-1} of sugar. Because of the similar structure of these sugars, supplying the same concentration yields effectively the same maximum density of bacteria. Six replicate flask communities were started for each sugar treatment. The bacteria were preconditioned during the prior day in the same medium with the sugar corresponding to their experimental treatment. Half of the flasks in each treatment were inoculated with REL606 and half with REL607, in an alternating design, so that any inadvertent cross-contamination of bacteria between the communities would likely be discovered by the difference in the arabinose-utilization marker. Small initial population sizes were used to ensure that mutations for resistance and host-range expansions evolved *de novo*. All cultures were kept at 37°C with shaking at 120 rpm. After 24 h, a random volumetric sample of 1% of the community was transferred to a flask with fresh medium and allowed to regrow. At the end of each 24-h period, two 1-mL samples of the flasks were preserved frozen at -80°C with 15% v/v glycerol. Population densities were determined each day by counting bacterial colonies on LB (Luria-Bertani) agar plates [34], and by counting phage plaques on lawns of REL606 [1]. Serial transfers and sampling were repeated for 25 days. Some populations stopped producing plaques, which we confirmed resulted from extinction of the phage by quantitative polymerase chain reaction (PCR) [33].

E. coli clones were isolated by picking individual colonies from the plates described above, streaking them on a fresh LB agar plate, allowing new colonies to form, and repeating the procedure once more to eliminate any possible phage carriage. Colonies were then selected from the second streak and grown in liquid LB overnight at 37°C and 120 rpm, and a sample was frozen in 1 mL with glycerol as described above. Phages were isolated by picking individual plaques and culturing them on REL606 using standard methods [1]. Phage isolates were stored at 4°C with 4% v/v chloroform added to maintain sterility.

20.2.3 Sequencing Resistance Genes

Candidate genes from clones sampled on days 3, 10, and 25 were sequenced to discover any resistance mutations. We targeted two loci: *lamB*, which encodes the receptor LamB; and *malT*, which encodes MalT, a transcriptional activator for *lamB* [15]. We examined one clone from each time-point from three of the six replicate populations for each treatment. We chose to use three replicates because only three phage populations survived for the full 25 days in glucose and maltose. We sequenced DNA fragments amplified by PCR and purified with GFX columns. An ABI sequencer run by Michigan State University's Research Technology Support Facility was used for sequencing. The primer sequences used to amplify *lamB* were 5' TTCCCGGTAATGTGGAGATGC 3' and 5' AATGTTTGCCGGGACGCTGTA 3' positioned at 1,398 bases upstream and 504 bases downstream of the protein-coding sequence. The primer sequences for amplifying *malT* were 5' CACCGGTTTGGCGAATGG 3' and 5' GCGGCGGTGGGGGAATA 3' at 424 bases upstream and 212 bases downstream of the coding sequence.

20.2.4 Competition Experiments to Determine Fitness Costs of Resistance

To determine whether maltodextrins led to the evolution of more costly resistance than did glucose, we competed a resistant clone isolated from each community against the ancestor with the opposite arabinose-utilization marker. The resistant clones were sampled on day three of the experiment. We chose this early time-point because preliminary experiments indicated that resistance had already evolved by then, and it was too soon for the bacteria to evolve additional mutations that might ameliorate the cost. Competitions were performed in the absence of phage by inoculating a flask with $\sim 1.25 \times 10^6$ preconditioned cells each of the resistant clone and the ancestor, allowing them to grow and compete, and quantifying their relative fitness as the ratio of their realized growth rates [25, 42]. We measured each clone's fitness in the same sugar environment in which it evolved, with each assay running

for three days (three 1:100 serial transfers), and with three replicate competition assays for every clone.

20.2.5 Experimental Test of Tradeoff-mediated Coexistence

We performed an experiment to test whether a tradeoff between resistance to phage and competitiveness for limiting resources was responsible for maintaining genetic variation in the bacterial populations, and if this variation in turn allowed phage λ to persist in the maltotriose environment. We selected two clones that evolved from REL606 (Ara^- marker state) with maltotriose as the limiting resource. Both were previously shown to have high levels of resistance to λ [16], but one of them, clone 19a, suffered a cost for this resistance in terms of a reduced growth rate on maltotriose, whereas the other one, clone 56a, did not [29]. We constructed communities with phage cI26, sensitive host strain REL607 (Ara^+ marker state), and one of the two resistant clones. If the tradeoff model for phage-mediated coexistence applies, then the sensitive host REL607 should decline when the resistant clone 56a is present, because the sensitive host has no growth advantage and yet can be infected by phage. The declining REL607 population should, in turn, cause the phage population to decline. By contrast, those communities that include the resistant clone 19a, which has reduced competitive fitness, should maintain populations of both the sensitive host REL607 and phage λ. Communities were initiated with $\sim 1,000$ particles of λ and ~ 500 cells each of the sensitive and resistant *E. coli* strains. We constructed six replicates with each resistant clone, and propagated the communities by serial transfer for four days in modified DM with maltotriose as the limiting sugar. Population densities were sampled at the end of each day. Phage populations were enumerated as before. The total bacterial populations (resistant and sensitive combined) were enumerated by counting colonies on LB agar plates; sensitive cells only were enumerated by counting colonies on minimal arabinose agar plates, where only the sensitive Ara^+ REL607 progenitors could form colonies.

Before running this experiment, we performed two additional checks. First, we confirmed that the differences in growth rate led to differences in competitive fitness between the two resistant hosts by running the previously described competition protocol using clones 19a and 56a, with a control in which the marked sensitive clones REL606 and REL607 competed against one another. Second, we analyzed the full genomes of the two resistant clones to determine if they had other mutations. To that end, the Research Technology Support Facility at Michigan State University sequenced the genomes using an Illumina Genome Analyzer IIx. To obtain genomic samples, we revived frozen bacteria in LB medium, grew them overnight, and isolated DNA from several mL of culture with Qiagen genome tips. The DNA samples were fragmented by sonication, labeled with bar-coded attachments, and run as multiplexed samples over four lanes. We called mutations from the resulting 75-base single-end reads using *breseq* version 0.13 [14] and using the REL606 genome [22] as the reference (GenBank accession: NC_012967.1).

20.3 Results

20.3.1 Population Dynamics Vary by Environment

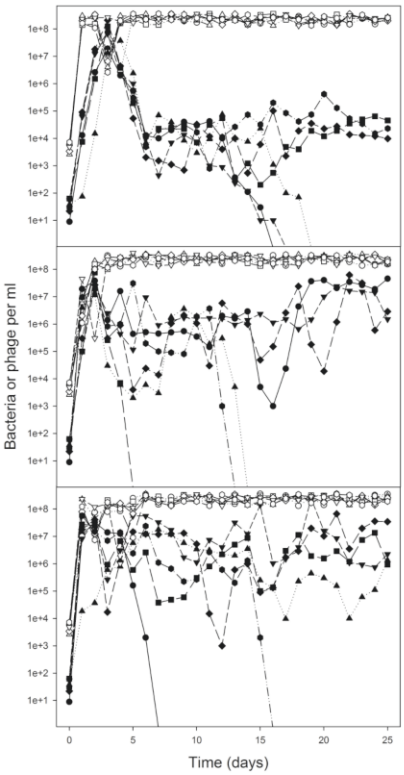


Fig. 20.2: Phage population dynamics (filled symbols) vary by treatment (top, glucose; middle, maltose; bottom, maltotriose), whereas bacterial dynamics (open symbols) do not. Each panel shows six replicate communities. The limit of phage detection was ∼3 per mL; the lines that cross the x-axis indicate extinctions. Except for day 0, densities were measured at the end of each 24-h cycle, just before 1% of the culture volume was transferred to a flask with fresh medium.

The phage populations went extinct in almost half of the communities by the end of the 25-day experiment (Fig. 20.2). Contrary to our expectation, the extinctions were not treatment specific; instead, phage survived in half of the populations in glucose, half in maltose, and two-thirds in maltotriose. We had predicted that λ would not survive in the glucose treatment because *E. coli* could evolve mutations that suppress expression of the LamB receptor, and do so without fitness costs be-

cause glucose enters cells by another pore. This absence of a tradeoff would allow resistant mutants to fix in the bacterial population and cause λ to decline to extinction.

Instead, however, a small but stable phage population of $\sim 10^4 \text{mL}^{-1}$ persisted for an extended period (Fig. 20.2, top panel). The phage were likely maintained as a consequence of so-called "leaky" resistance, in which a subpopulation of sensitive cells is continuously generated from largely resistant cells, probably by occasional spontaneous induction of LamB production, despite mutations in the gene that encodes the MalT activator [10, 28]. The phage populations, prior to extinction in some cases, were generally larger and experienced greater fluctuations in the two maltodextrin treatments (Fig. 20.2, middle and bottom panels). By contrast, the bacterial populations remained high and stable in all populations in all three treatments.

20.3.2 Fitness Costs of Resistance Vary by Environment

In the glucose environment without λ present, the six evolved resistant clones showed little or no loss of competitive fitness, with no change on average (Fig. 20.3). In maltose, by contrast, all six resistant clones showed reductions in their competitive fitness. The average fitness reduction in the maltotriose-evolved clones was similar to those that evolved in maltose, although the clones from the maltotriose environment showed greater variance in fitness. Owing to this heterogeneity in maltotriose, the differences among the three sugar treatments were only marginally significant (Kruskal-Wallis test, $p < 0.05$), with that result driven largely by the difference between the glucose and maltose treatments (Mann-Whitney test, $p < 0.01$). On balance these data imply that resistance typically imposed a substantial fitness cost when the bacteria evolved on maltodextrins, but not on glucose, consistent with our expectations.

20.3.3 Resistance Mutations Vary by Environment

We sequenced two candidate genes, *lamB* and *malT*, to find mutations responsible for the resistance to phage λ that evolved in all three of the sugar environments (Table 20.1). In the glucose treatment, we found many mutations in *malT*, which encodes MalT, a transcriptional activator of genes involved in maltodextrin uptake and metabolism, including LamB, which is the receptor for phage λ (Fig. 20.1). These gene products are not useful for growth on glucose, and so the *E. coli* in this treatment were able to achieve high levels of resistance without an associated fitness cost by disrupting this activator and turning off expression of LamB. Also, the *malT* gene is about three times as long as *lamB*, which exposes more sequence to mutations and may thus explain why it was mutated more often than *lamB*. Additionally,

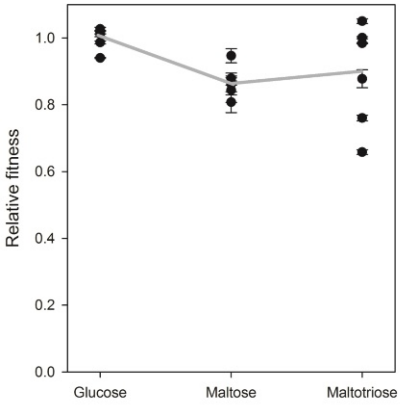


Fig. 20.3: Fitness of λ-resistant *E. coli* clones that evolved in three resource environments. Each resistant clone competed in the environment where it evolved against the sensitive ancestor with the opposite arabinose-utilization marker state. Each point shows the mean of three competition assays; error bars indicate standard errors, which in some cases are hidden by the symbols. One clone was examined from each independently evolved community (six from each treatment). The gray line connects the grand mean fitness values for the clones from each environment.

malT has an enigmatic 25-bp DNA sequence that has an elevated rate of duplication [10, 28].

In the maltose and maltotriose treatments, mutations did not evolve in the *malT* gene. Such mutations would reduce or eliminate expression not only of LamB, but also other proteins used to metabolize these two sugars after they enter the cell (Fig. 20.1). Instead, mutations evolved repeatedly in *lamB* in these two environments (Table 20.1). In maltose, all of the observed *lamB* mutations were frameshift or nonsense mutations, which presumably eliminated any functional LamB protein and thereby conferred high-level resistance to phage λ. These mutations are costly in maltose because LamB is the main pore protein used to move maltose across the outer cell envelope. However, such loss-of-function mutations are not lethal because the OmpF pore protein provides an alternative, though less efficient, route.

In the maltotriose treatment, by contrast, most mutations in *lamB* were point mutations, which changed the amino-acid sequence of LamB. Five of the six point mutations affected protein loops and probably interfered with λ binding (Table 20.1; position 856 is the exception). These mutations affected different loops, which might explain why maltotriose-evolved clones vary so much in their fitness costs (Fig. 20.3). The LamB pore is also larger than the OmpF pore, and maltotriose is larger than maltose, which may explain why loss-of-function mutations were less common in the maltotriose environment. Two of the *lamB* alleles sampled in the maltotriose treatment at the end of the experiment (day 25) had two separate muta-

tions each, suggesting a multi-step arms race between phage λ and the *lamB* gene in the *E. coli* hosts.

In all three sugar treatments, some clones that were resistant to phage λ had no mutations in either *lamB* or *malT* (Table 20.1). These clones presumably had mutations in other genes that can confer resistance [8, 27, 28].

Table 20.1: Mutations found by sequencing two candidate genes, *malT* and *lamB*, in λ-resistant clones sampled after 3, 10, and 25 days in three replicate communities of each treatment. The notation "unknown" indicates that no mutations were found; however, these clones presumably had mutations in other genes that conferred resistance.

Condition	Replicate population	Day	Gene mutated	Nucleotide position*	Mutation	Effect
Glucose	-3	3	*malT*	992	25 base duplication	frameshift
Glucose	+1	3	*malT*	912	△1 base duplication	frameshift
Glucose	+3	3	unknown			
Glucose	-3	10	unknown			
Glucose	+1	10	unknown			
Glucose	+3	10	*malT*	992	25 base duplication	frameshift
Glucose	-3	25	*malT*	2681	$T \longrightarrow C$	Leu $\longrightarrow$ Pro
Glucose	+1	25	unknown			
Glucose	+3	25	*malT*	2177	$A \longrightarrow T$	Asp $\longrightarrow$ Val
Maltose	-1	3	*lamB*	1162	△29 base duplication	frameshift
Maltose	-2	3	*lamB*	1162	△11 base duplication	frameshift
Maltose	+1	3	*lamB*	308	△11 base duplication	frameshift
Maltose	-1	10	unknown			
Maltose	-2	10	*lamB*	966	△11 base duplication	frameshift
Maltose	+1	10	*lamB*	429	$C \longrightarrow G$	stop
Maltose	-1	25	unknown			
Maltose	-2	25	*lamB*	966	△11 base duplication	frameshift
Maltose	+1	25	*lamB*	429	$C \longrightarrow G$	stop
Maltotriose	-2	3	*lamB*	697	△11 base duplication	frameshift
Maltotriose	-3	3	unknown			
Maltotriose	+1	3	*lamB*	1128	$G \longrightarrow T$	Trp $\longrightarrow$ Cys
Maltotriose	-2	10	*lamB*	709 856	$G \longrightarrow T$ $T \longrightarrow A$	Gly $\longrightarrow$ Cys Leu$\longrightarrow$Gln
Maltotriose	-3	10	*lamB*	509	$G \longrightarrow C$	Arg $\longrightarrow$ Pro
Maltotriose	+1	10	unknown			
Maltotriose	-2	25	*lamB*	716	$T \longrightarrow A$	stop
Maltotriose	-3	25	*lamB*	796 1128	+18 bases $G \longrightarrow T$	+6 amino acids Leu$\longrightarrow$Pro
Maltotriose	+1	25	unknown			

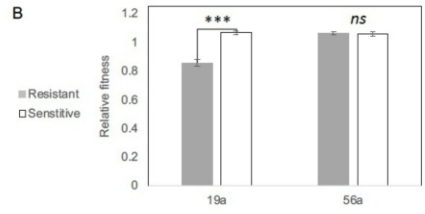
20.3.4 Experimental Test of Tradeoff-mediated Coexistence

There are multiple hypotheses for how populations of bacteria and lytic phage can coexist, despite their strong antagonism. Previous work has shown that λ persists in the glucose condition, despite *E. coli*'s ability to evolve high levels of resistance, because that resistance is often leaky [10, 28]. In particular, resistant cells with mutations in *malT* experience occasional spontaneous induction of LamB expression, thereby generating a small population of phenotypically sensitive cells that can sustain a small phage population. However, phage populations were much larger in the two maltodextrin treatments (Fig. 20.2) and, moreover, the evolved bacterial resistance in those communities was not caused by *malT* mutations (Table 20.1). These facts suggest that a different mechanism supports the λ population in those treatments. The coexistence hypothesis that has received the most attention in the literature is based on a tradeoff in bacteria between resistance and competitive fitness [4, 23, 24]. Such tradeoffs can prevent resistant mutants from sweeping to fixation, thus allowing the maintenance of a genetically sensitive host population, which in turn can support the phage.

Given the evidence for this tradeoff in maltodextrins (Fig. 20.2), we tested this hypothesis as follows. We constructed two types of synthetic communities in the maltotriose environment, each with three players: the ancestral λ, the sensitive *E. coli* ancestor, and an evolved resistant clone. The two types of community differed, however, in the identity of the resistant clone. Both clones had mutations in the *lamB* gene (Fig. 20.4A). One of them had a nonsense mutation in *lamB* and suffered a severe reduction in fitness in the absence of phage; the other resistant clone had an insertion mutation in that gene and showed no measurable fitness cost (Fig. 20.4B). The total bacterial population size at the end of this four-day experiment was unaffected by which resistant clone was used in these synthetic communities (Fig. 20.4C). However, as predicted by the tradeoff hypothesis, both the sensitive bacterial population (Fig. 20.4D) and the phage population (Fig. 20.4E) were significantly larger in the communities with the costly resistance than in those where the resistant bacteria were as good as their sensitive counterparts in competing for resources.

A

Strain	genome position	mutation	annotated	gene
19a	3,894,997	Δ3,873 bp	IS150-mediated	*rbsD–rbsB*
	4,227,513	+T	coding (611/1341 nt)	*lamB*
56a	1,630,681	+9 bp	IS1-mediated coding (422-430/1020 nt)	*rspB*
	4,227,698	Δ18 bp	coding (796-813/1341 nt)	*lamB*



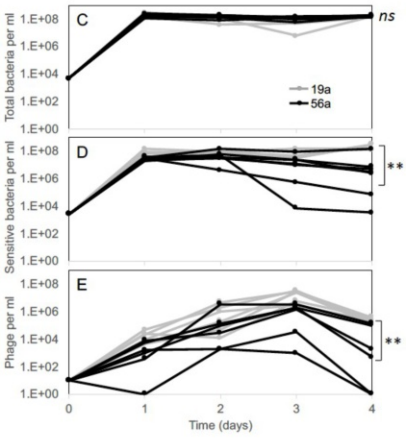


Fig. 20.4: Coexistence of phage λ and *E. coli* in the maltotriose environment depends on the tradeoff between resistance and competitive fitness in the absence of phage. (A) Mutations in two resistant clones, based on whole-genome sequencing. (B) Clone 19a has lower fitness than its sensitive ancestor, whereas clone 56a does not. (C) Total bacterial density is similar whether the communities include the 19a (gray lines) or 56a (black lines) resistant clone. (D) The final density of the sensitive ancestral strain is lower in the presence of the high-fitness resistant clone 56a (black lines) than in presence of the low-fitness clone 19a (gray lines). (E) The final density of phage λ is also lower in the presence of 56a (black lines) than 19a (gray lines); a density of 1 indicates the phage population was below the limit of detection. Significance levels are based on *t* tests in (B) and Mann-Whitney tests in (C), (D), and (E), with results shown as follows: ns, not significant; $P < 0.01^{**}$; $P < 0.001^{***}$.

Genome sequencing of these resistant clones revealed that each had another mutation in addition to the one in *lamB*. These mutations were an insertion and a deletion, both caused by IS elements (Fig. 20.4A). Neither affected gene is known to confer resistance to λ [27]. This type of mutation occurs at a high rate in many bacteria including this strain of *E. coli* [10, 11, 37], and these mutations were probably inconsequential hitchhikers without major phenotypic effects.

20.4 Discussion

We examined the effects at several levels – from genomes to community structure – of a subtle change in the environment. In particular, we provided otherwise identical model communities of one lytic phage and one bacterial species with "glucose" in three forms: glucose, maltose, and maltotriose. Maltose and maltotriose are sugars with two and three linked glucose moieties, respectively, each involving one additional covalent bond.

Despite their fundamental similarity, these treatments generated distinct eco-evolutionary dynamics. These differences follow from the fact that *E. coli* expresses a different pore protein, LamB, which allows the larger maltose and maltotriose molecules to cross its outer membrane more efficiently than does the smaller-diameter pore protein, OmpF, which suffices for glucose (Fig. 20.1). In glucose, the populations of *E. coli* typically evolved resistance by mutations in a regulatory gene called *malT*, severely reducing the expression of LamB, which phage λ also uses to enter host cells. These *malT* mutations had no fitness costs for the bacteria when growing on glucose, and the phage populations declined following their initial expansion. The phage also appeared unable to readily evolve counter-defenses. Nonetheless, half of the phage populations persisted because this mode of resistance was phenotypically leaky, such that LamB production was spontaneously induced in a small fraction of the resistant mutants.

In the maltose and maltotriose treatments, by contrast, mutations in *malT* that inactivate LamB expression would be extremely deleterious to the bacteria. Such mutations would not only reduce the protein used for efficient acquisition of these larger sugars, they would also reduce the enzymes used in the first steps of metabolizing these sugars (Fig. 20.1). Instead, in these treatments the bacteria typically evolved resistance via mutations in *lamB*, the gene that encodes the LamB pore and receptor used by phage λ. In the case of the maltose environment, many of the *lamB* mutations were frameshift and nonsense mutations, which presumably eliminated any functional LamB pores. These mutations were quite costly to the bacteria in terms of competitive fitness for maltose, but some intermediate-sized maltose molecules could nonetheless enter cells via the smaller OmpF pores (Fig. 20.1). Evidently, reduced competitiveness was worth it when faced with the lethal consequences of phage infection. As a result of this tradeoff, a minority population of genetically sensitive cells (as opposed to leaky resistant cells) could persist and, in turn, sustain a phage population.

In the maltotriose environment, the OmpF pores are of little use in allowing such large molecules to enter the cell, and the LamB protein is even more important than it is in maltose. Mutations that destroy LamB were not tolerated in this treatment. Instead, we typically saw point mutations that altered the structure of the protein in ways that interfered with the phage's ability to adsorb to the altered protein, but evidently still allowed maltotriose to enter the cell. These mutations had variable effects on competitive fitness, which in turn affected whether sensitive cells and phage could persist (Fig. 20.4). Moreover, the subtle changes in LamB structure appear to have led to an evolutionary response by phage λ, which in turn sometimes led to further changes in LamB, indicating a multi-step coevolutionary arms race.

20.5 Coda

Has the reader ever left an ecology seminar discouraged because the presenter identified yet another variable that matters for some ecosystem property or dynamics?

For readers who are not ecologists, this feeling is similar to the exhaustion experienced after seeing yet another headline describing supposed health benefits from some dietary supplement. An overarching lesson from much of life-sciences research is that subtle perturbations often matter for the health of biological systems, whether individual organisms or whole ecosystems. Complexity theory provides an explanation for why living systems are so sensitive, and therefore why it is difficult to make predictions in biology. Our results, however, should not be interpreted as just another example of identifying a variable that makes it difficult to predict ecological and evolutionary dynamics. Instead, our study offers a ray of hope for prediction in the face of biocomplexity. By leveraging information gained from microbial genetics and molecular biology, we were able to identify in advance the proverbial "needle in a haystack" of chemical bonds that would significantly impact the ecology and evolution of an experimental community. This work shows that integrating molecular knowledge with population-biology models can improve the predictive power of biological theories.

Acknowledgements We thank Erik Goodman for his outstanding leadership of BEACON. This work was supported, in part, by the BEACON Center for the Study of Evolution in Action (National Science Foundation Cooperative Agreement DBI-0939454) and the Defense Advanced Research Projects Agency (HR0011-09-1-0055). We thank Anurag Agrawal, Jeff Barrick, Tom Ferenci, Mike Wiser, and Luis Zaman for helpful discussions, Neerja Hajela for help with lab work, and Donald Court for sharing phage cI26. J.R.M. will make the evolved strains available to qualified recipients, subject to completion of a material transfer agreement (http://blink.ucsd.edu/research/conducting-research/mta/index.html).

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