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PARALLEL CHANGES IN HOST RESISTANCE TO VIRAL INFECTION DURING 45,000 GENERATIONS OF RELAXED SELECTION

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The dynamics of host susceptibility to parasites are often influenced by trade-offs between the costs and benefits of resistance. We assayed changes in the resistance to three viruses in six lines of *Escherichia coli* that had been evolving for almost 45,000 generations in their absence. The common ancestor of these lines was completely resistant to T6, partially resistant to T6* (a mutant of T6 with altered host range), and sensitive to λ. None of the populations changed with respect to resistance to T6, whereas all six evolved increased susceptibility to T6*, probably ameliorating a cost of resistance. More surprisingly, however, the majority of lines evolved complete resistance to λ, despite not encountering that virus during this period. By coupling our results with previous work, we infer that resistance to λ evolved as a pleiotropic effect of a beneficial mutation that downregulated an unused metabolic pathway. The strong parallelism between the lines implies that selection had almost deterministic effects on the evolution of these patterns of host resistance. The opposite outcomes for resistance to T6* and λ demonstrate that the evolution of host resistance under relaxed selection cannot be fully predicted by simple trade-off models.

KEY WORDS: Adaptation, bacteriophage T6, bacteriophage lambda, cost of resistance, *Escherichia coli*, experimental evolution, long-term evolution experiment, trade-offs.

The evolutionary dynamics of populations' resistance to their enemies have long been of interest (Ehrlich and Raven 1964; Van Valen 1973; May and Anderson 1983). The costs and benefits of resistance, especially coupled with spatiotemporal variation in selection exerted by natural enemies, can maintain genetic variation in host populations (Gillespie 1975; Thompson 2005). Both historical and experimental approaches have been pursued to study these dynamics, although there have been few long-term studies.

Two comparative approaches have often been employed to study the evolution of resistance. First, populations living on

islands or otherwise protected from their natural enemies were predicted to become more susceptible to those enemies, given relaxed selection for resistance and its presumed cost (Brodie 1991; Bowen and Van Vuren 1997; Stastny et al. 2005; Kato et al. 2008). These studies have frequently reported reduced resistance in the absence of enemies, although these studies typically lack evolutionary replication because they focus on a single host lineage. Second, the patterns of sequence variation at loci conferring resistance have been used to infer evolutionary dynamics (Stahl and Bishop 2000). Positive selection for resistance in the face of a coevolving pathogen, for example, has been inferred from observing an excess of nonsynonymous to synonymous substitutions in genes controlling resistance to infection.

Experimental approaches, including quantitative genetics, have also provided a way to assess the costs and benefits of resistance using single-generation performance assays and selection experiments (Simms 1992; Stowe 1998; Shonle and Bergelson 2000). However, multigenerational studies are needed to understand the long-term dynamics of resistance evolution because responses to parasite-mediated selection may be ameliorated or intensified by other ecological interactions (e.g., competition), by pleiotropic effects of resistance genes, and by selection at other life stages than those measured in short-term assays.

Experimental microcosms have proven to be powerful for the study of resistance evolution (Abedon 2009). For example, an interplay between the benefits and costs of resistance by algae to rotifers promoted rapid evolution and, moreover, substantially influenced their predator–prey cycles (Yoshida et al. 2003, 2004; Meyer et al. 2006). In various experiments with *Pseudomonas* bacteria, costs of resistance, predator–prey evolutionary dynamics, and community consequences have been well-studied (Buckling et al. 2006; Poullain et al. 2008; Abedon 2009; Escobar-Paramo et al. 2009). Although costs of resistance are often detected, they are sometimes quite small, they may be ameliorated by selection (Lythgoe and Chao 2003), and they may vary depending on other genetic factors (Buckling et al. 2006).

The evolution of resistance of the bacterium *Escherichia coli* to several viruses that infect it has also been well-studied using chemostats (Lenski and Levin 1985; Lenski 1988a; Bohannan and Lenski 2000a). In these studies, resistance is usually discrete and qualitative (i.e., complete resistance or susceptibility), the mechanisms of resistance are well-characterized, and the multigenerational fitness consequences of resistance have been investigated. For example, Lenski (1988b,c) studied the evolution of resistance to bacteriophage T4. Resistance was initially very costly, although the magnitude of this cost varied among independent host mutants. After 400 generations of evolution in the absence of T4, however, the fitness of resistant lines had achieved nearly the same level as susceptible populations that were evolving under identical conditions. Interestingly, the resistant populations did not revert to susceptibility, but instead they had evolved compensatory changes that ameliorated the cost of resistance.

The goal of the present study is to examine the long-term fate of resistance in host populations in which the resistance is unnecessary owing to the absence of parasites. We take advantage of an experiment, started over 20 years ago with 12 replicate populations of *E. coli* (Lenski et al. 1991; Lenski and Travisano 1994; Cooper and Lenski 2000). Based on simple trade-off models, we initially predicted the loss of resistance by these bacteria to viruses, or phages, that infect them. However, our increased mechanistic understanding of the costs of resistance and pleiotropic effects of certain mutations that confer resistance altered our expectations for three different phages, as described below. The ancestral strain was resistant to the lytic phage T6, and the replicate populations have evolved independently for nearly 45,000 generations in the absence of T6 or any other parasites. If resistance to T6 imposed even a small cost, then one would expect this resistance to decay in a convergent manner (i.e., across the replicate lines) over time. We test that prediction by reviving frozen stocks of six of these lines from several time points and assaying their resistance to phage T6. We also directly assessed the cost of resistance to T6 using reverse genetics to create isogenic resistant and susceptible *E. coli* strains. In the course of our preliminary work with T6, we discovered a mutant phage, which we call T6*, that can infect the ancestral *E. coli* strain, although its infectivity on the ancestor was much lower than it was on another T6-sensitive host. We therefore also examined the evolution of susceptibility to T6*, where that trait was evidently quantitative rather than discrete.

A third phage, called λ, is unrelated to the other two, but offers an additional contrast in that the ancestral strain was fully sensitive to this phage. Moreover, from previous research on *E. coli* (Schwartz 1987; Wang et al. 1997), and these evolving lines in particular (Pelosi et al. 2006), we expected that the expression of the receptor protein exploited by λ to infect cells may have evolved. In particular, the LamB surface protein is part of a regulon that *E. coli* uses to transport and metabolize maltose and other maltodextrin sugars that were also not present in the environment during the evolution experiment. Indeed, proteomic and genetic analyses revealed that reduced expression of this regulon, including the LamB protein, had evolved in some of these long-term lines (Pelosi et al. 2006), although the consequences of those changes for resistance to λ were not analyzed. We therefore examined the evolution of susceptibility to phage λ because it offered a potentially instructive counterexample to the intuition that parasite resistance should decline under relaxed selection.

Materials and Methods

THE LONG-TERM EVOLUTION EXPERIMENT

Detailed information on the long-term evolution experiment (LTEE) can be found elsewhere (Lenski et al. 1991; Lenski and Travisano 1994; Lenski 2004). Briefly, two ancestral clones, called REL606 and REL607, were used to found six populations each in 1988. These clones differ by a selectively neutral mutation in *araA* that serves as an easily scored marker in competition assays based on the arabinose-utilization phenotype. The 12 populations have been serially propagated for over 20 years in a glucose-limited medium. Each day, 0.1 mL is removed from the population and transferred into 9.9 mL of fresh medium, where it grows until the glucose is depleted. Given the 1:100 daily dilution and regrowth, the populations undergo ~6.6 ($=\log_2 100$) generations of binary fission each day, and they had achieved some 44,500 generations at the time that we commenced this project.

Every 500 generations (75 days), a sample of each population was stored frozen at −80°C with glycerol, which serves as a cryoprotectant. These samples include essentially all the diversity present in each population at the corresponding generation, because they were obtained by adding glycerol to, and preserving, the majority of the population from which the transfer was made. For our analyses, we revived bacteria from six populations using samples taken at 0, 2000, 10,000, 20,000, 30,000, 40,000, and 44,500 generations. Three clones (asexual genotypes) were randomly isolated from each sample to assess their resistance phenotypes. In choosing the populations for this study, we excluded some that had evolved to grow poorly and inconsistently on our experimental plates.

As noted in the Introduction, the ancestral strain used for the LTEE was resistant to phage T6 (Lenski et al. 1991). That resistance reflects a spontaneous mutation that had been selected by a geneticist using an even earlier progenitor (Lederberg 1966; Daegelen et al. 2009). In particular, a point mutation generated a premature stop codon in the *tsx* gene (Manning and Reeves 1976; Bremer et al. 1990; Studier et al. 2009), which encodes an outer membrane protein that is the target for adsorption of phage T6 (Schneider et al. 1993). The mutation confers complete resistance to "wild type" T6, but it provides only partial (quantitative) resistance to some extended host-range mutants of T6, as has been reported for certain other T6-resistant mutants (Luria 1945). One of us (REL) obtained a stock of phage T6 many years ago from Bruce Levin (now at Emory University). For this study, we also isolated a host-range mutant from a rare plaque (zone of lysis) that originally formed on a lawn of a T6-resistant clone sampled at generation 44,500 from the Ara + 5 population of the LTEE. We refer to this mutant phage as T6*.

The ancestral strain in the LTEE was sensitive to λ infection. Phage λ targets the outer-membrane porin LamB, which the cell uses to transport maltose and other maltodextrin sugars across the outer membrane into the periplasmic space, after which another system actively transports these sugars into the cell (Wang et al. 1997). Wild-type λ is a lysogenic phage that sometimes becomes incorporated into the host chromosome, which would unnecessarily complicate our analysis of changes in host resistance. Therefore, we used a strictly lytic mutant cI26 (commonly referred to as λ*vir*), provided by Donald Court (National Cancer Institute). This mutant phage harbors at least one chemically induced mutation in the *cI* gene, which encodes a repressor protein required for lysogeny. This phage has the same mode of entry into the cell as wild-type λ, but a successful infection is lethal to the host cell. For simplicity, we refer to this mutant phage as λ.

ASSAYS OF BACTERIAL SUSCEPTIBILITY

We assessed susceptibility to phage infection using "spot" plates (Adams 1959), on which a dense lawn of bacteria is immobilized in a matrix of soft agar. An aliquot of a liquid stock of phage is then spotted on the bacterial lawn and incubated overnight. If a virus infects a cell, and if that infection propagates to other cells, then a spot of clearing, known as a plaque, will occur where the cells have lysed in the otherwise turbid lawn of cells. This approach has been widely used for decades to quantify differences among bacterial strains in their susceptibility to phage infection, although a recent study described some potential problems with this inference (Loś et al. 2008). These problems were not an issue for our study, however, as we confirmed our results on differences in susceptibility using a second method, in which liquid cultures of bacteria were challenged with phage (see Supporting Information).

The spot assays were set up by pouring a mix of 2 mL of molten soft agar and 1 mL of *E. coli* culture over a Petri plate containing a base of hardened Luria Bertani (LB) agar. Cultures were grown overnight at 37°C and shaken at 120 rpm in liquid LB medium [see Sambrook et al. (1989) for media recipes]. After the soft agar had solidified, six evenly spaced 10-μL spots of a single phage lysate were spotted onto the top agar. Each spot after the first represented a further 100-fold dilution of the phage lysate; thus, the first spot has the original phage concentration, followed by relative concentrations of 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10}. Phage infectivity can vary over many orders of magnitude, and the idea of the spot test is to include a dilution that allows some countable number of discrete plaques, such that host susceptibility can be quantified. Phage lysates were standardized such that a 10-μL aliquot of the 10^{-6} dilution yielded ~100 plaques (10^{10} plaque-forming units per milliliters) when tested on a lawn of an *E. coli* K-12 strain (JA221 from Moyed et al. 1983) that is sensitive to phages T6, T6*, and λ. The plates were incubated

overnight at 37°C, and the plaques within each appropriate spot were counted.

Assays were performed on 108 evolved clones, including three clones from each of six populations (those designated Ara − 3, Ara − 5, Ara + 1, Ara + 2, Ara + 4, and Ara + 5) at six time points (generations 2000, 10,000, 20,000, 30,000, 40,000, and 44,500). In addition, three clones were isolated from the marked Ara^+ and Ara^- ancestral variants to measure the ancestor's susceptibility to the phage. Assays were conducted in a completely randomized design, with two replicate assays for each clone that were averaged to produce a single value for the statistical analyses. The susceptible K-12 strain was also included in the assays to verify the infectivity of the phage lysates, but it was not included in the statistical analyses. We performed the T6 and T6* assays on the same day, whereas the λ assays were performed on a later date. All aspects of the assays, including the *E. coli* clones tested, were identical for the three phages.

We analyzed the susceptibility assays separately for each phage by performing a mixed-model ANOVA using restricted maximum-likelihood (JMP, version 7). Time (generation) was treated as a continuous effect, whereas line (population) and the line-by-time interaction were random effects. Random effects were tested using likelihood ratios (Littell et al. 1996).

SEQUENCING THE *tsx* AND *malT* GENES

We sequenced the *tsx* gene (GenBank accession number: 8178898), which encodes the receptor for T6 adsorption, in the ancestor of the LTEE, in three clones from each of the six lines at 40,000 generations, and in the T6-sensitive *E. coli* K-12 strain. Primers (5′ATCCCGGCATTTTCATAA3′ and 5′AAGGGGATT TCTGTGGAT3′) were designed to cover all of *tsx* as well as 150 base pairs upstream and 129 base pairs downstream of the gene. The PCR products were then purified on a GFX column, and *tsx* and the adjacent sequences were obtained in their entirety for each clone using an ABI automated sequencer. Sequences were aligned using DNA Star SeqMan (version 1) and inspected visually for any differences.

We also sequenced the *malT* gene (GenBank accession number: 8177524) in clones sampled at 40,000 generations from two of the six LTEE lines, Ara − 5 and Ara + 5, as well as the ancestral strain. The *malT* gene encodes the transcriptional activator of the maltose regulon, which includes the *lamB* gene that encodes the LamB surface protein that phage λ targets in sensitive cells. Previous work had identified mutations in *malT* in several of the LTEE lines (Pelosi et al. 2006), and we sought to determine whether some of the changes we saw in resistance to λ might also involve mutations in this gene. Primers 5′CACCGGTTTGGCGAATGG3′ and 5′GCGGCGGTGGGGGAATA3′ were designed to cover all of *malT* as well as 392 and 211 base pairs upstream and downstream, respectively. The subsequent steps proceeded as above for the *tsx* gene.

DIRECT TEST OF THE COST OF RESISTANCE

The long-term evolutionary fate of resistance is likely to depend strongly on how costly resistance is in the absence of selection for that phenotype. Therefore, we used reverse genetics to construct a strain, in the ancestral background, that is isogenic except for the mutation that confers resistance to phage T6. We then used this isogenic strain in competition assays to measure directly the cost of resistance to T6.

To produce a T6-sensitive variant of the Ara^- ancestor, we replaced its *tsx* gene containing the premature stop codon with the wild-type version of that gene by using the "gene-gorging" procedure described by Herring et al. (2003) and modified by Sleight et al. (2008). Primers 5′TAGGGATAACAGGGTAATGCTGT TCCCGCGAGTTTGT3′ and 5′GGATGCGCCGGTATTCTTC3′ were designed to flank 1000 bp around the premature stop at codon 258 (out of 295) and used to PCR-amplify a fragment from *E. coli* K-12 (strain JA221) that has a wild-type (susceptible) version of *tsx*. This fragment was incorporated into a TOPO10 plasmid and used to transform the LTEE ancestor (strain REL606) together with a second plasmid overexpressing the I-*Sce*I restriction enzyme (Herring et al. 2003). We then screened 800 transformed clones for sensitivity to T6 using spot plates; only two of the clones were sensitive. The transformed strain that we would use in experiments to assess the cost of resistance was designated JRM100, and the success of the allelic exchange was confirmed by sequencing the entire *tsx* gene. The only change was at the site that had encoded the premature stop codon. JA221 has one other difference in *tsx* from REL606, a synonymous change in codon 88. However, this synonymous difference was not integrated into the T6-sensitive JRM100.

We then measured whether there was any difference in fitness by competing T6-sensitive and T6-resistant strains under the same phage-free conditions as used during the LTEE (Lenski et al. 1991). The arabinose-utilization marker was used to distinguish the competitors. In particular, the Ara^- T6-resistant ancestor REL606 and its T6-sensitive counterpart JRM100 competed separately against the Ara^+ T6-resistant ancestor REL607, each with 10-fold replication. These pairwise competitions were propagated for 6 days, with plating onto tetrazolium-arabinose indicator agar on days 0, 1, 3, and 6 to determine the relative abundance of the competitors. We then calculated the relative fitness of the competitors from those data, where relative fitness is calculated as the ratio of net growth rates achieved during their direct competition (Lenski et al. 1991), and we compared the fitness values estimated for the otherwise isogenic T6-sensitive and T6-resistant strains.

Pelosi et al. (2006) performed similar assays to determine the fitness effect of mutations in the *malT* gene, also in the ancestral

genetic background and culture conditions of the LTEE. They found that the two mutant strains they studied each had small, but significant, advantages over their sensitive counterpart, even though no phage were present in the cultures.

We were unable to measure directly the cost of resistance to T6* because we do not know which loci harbor the mutations responsible for the quantitative variation in resistance to this phage. Hence, we cannot make and compete otherwise isogenic strains that differ only by the relevant mutations.

Results

All of the clones that we tested from all populations and generations appeared to be completely resistant to the wild-type T6 phage. However, by using a high-density phage T6 lysate, we observed a few plaques on the evolved bacteria. We isolated phage from six of these plaques, and all six of these new lysates consistently produced plaques on the T6-resistant ancestor as well as on the evolved bacteria, indicating that they were host-range mutants. We chose one of these host-range mutants, designated T6*, for further analysis. We infer that most or all of the plaques we observed when we challenged the evolved bacteria with the wild-type T6 lysate were produced by spontaneous host-range mutants within that lysate. There is no compelling evidence, therefore, that the evolved bacteria have increased susceptibility to the wild-type phage.

The sequence of the *tsx* gene that encodes the receptor for the wild-type T6 phage was identical in the T6-resistant ancestor of the LTEE and in a T6-sensitive *E. coli* K-12 strain, with the exception of two mutations, one of which is a synonymous substitution. The mutation that confers resistance is a premature stop at codon 258 of 295; this effect was confirmed by replacing that codon with the corresponding one from the K-12 strain. The resulting construct was just as sensitive to T6 as K-12 (see Table S1). None of the 18 clones we sampled from the six evolved lines at generation 40,000 had any other mutations in *tsx* or the adjacent regions that we sequenced.

This result ran counter to our expectation that the bacteria would evolve increased sensitivity to T6, perhaps even by reverting the mutation in *tsx* that made them resistant, in the prolonged absence of any phage. Considering the duration of the evolution experiment and the corresponding opportunity for slightly beneficial alleles to fix, there was substantial potential to ameliorate even a small cost to resistance. However, resistance to T6 was not costly in the environment of the LTEE. In fact, the resistance caused by the premature stop codon in the *tsx* gene turns out to confer a slight benefit during competition in the phage-free environment of the evolution experiment (Fig. 1). The T6-resistant ancestor was ~1.3% more fit than its otherwise isogenic

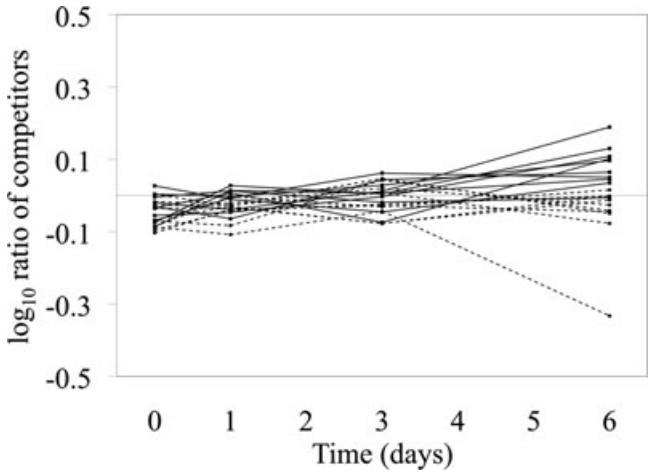

Figure 1. **Log_{10} ratios of competing pairs of *E. coli* strains across six daily transfer cycles. For solid and dashed lines, the competitors of interest (reflected in numerator counts) are, respectively, the Ara^- T6-resistant ancestor and its T6-sensitive derivative. For both the solid and dashed lines, the common competitor (reflected in dominator counts) is an Ara^+ mutant of the T6-resistant ancestral strain. Note that the resistant competitor of interest was relatively more abundant than its sensitive counterpart at the end of most assays, but not at the beginning, indicating a slight competitive disadvantage to being sensitive even in the absence of the phage. See text for statistics.**

T6-sensitive counterpart ($t = 3.81$, df = 18, two-tailed $P = 0.0013$). Thus, the maintenance of T6-resistance for almost 45,000 generations of the evolution experiment is consistent with the absence of any associated cost of resistance.

By contrast, all six evolving lines showed substantial increases in susceptibility to the host-range mutant phage T6* over this same period (Fig. 2). The temporal trend was significant, as was the variation among the lines in susceptibility (Table 1). However, the line-by-time interaction was not significant in our

Table 1. **Statistical analysis of the effects of evolved line (six lineages started from the same ancestor), time (seven points from 0 to 44,500 generations), and their interaction on the susceptibility of *E. coli* populations to parasitism by phages T6* and λ. Three clones were tested from each line at each time point. Time is a fixed effect and was evaluated using an *F* test; line and the interaction term are random effects, and they were evaluated by likelihood ratio tests.**

	Phage T6*			Phage λ		
	Df	*F* or χ^2	*P*	Df	*F* or χ^2	*P*
Time	1,5.89	21.93	0.004	1, 6.19	12.95	0.011
Line	1	5.33	0.010	1	34.71	<0.001
Line×Time	1	0.34	0.280	1	18.30	<0.001

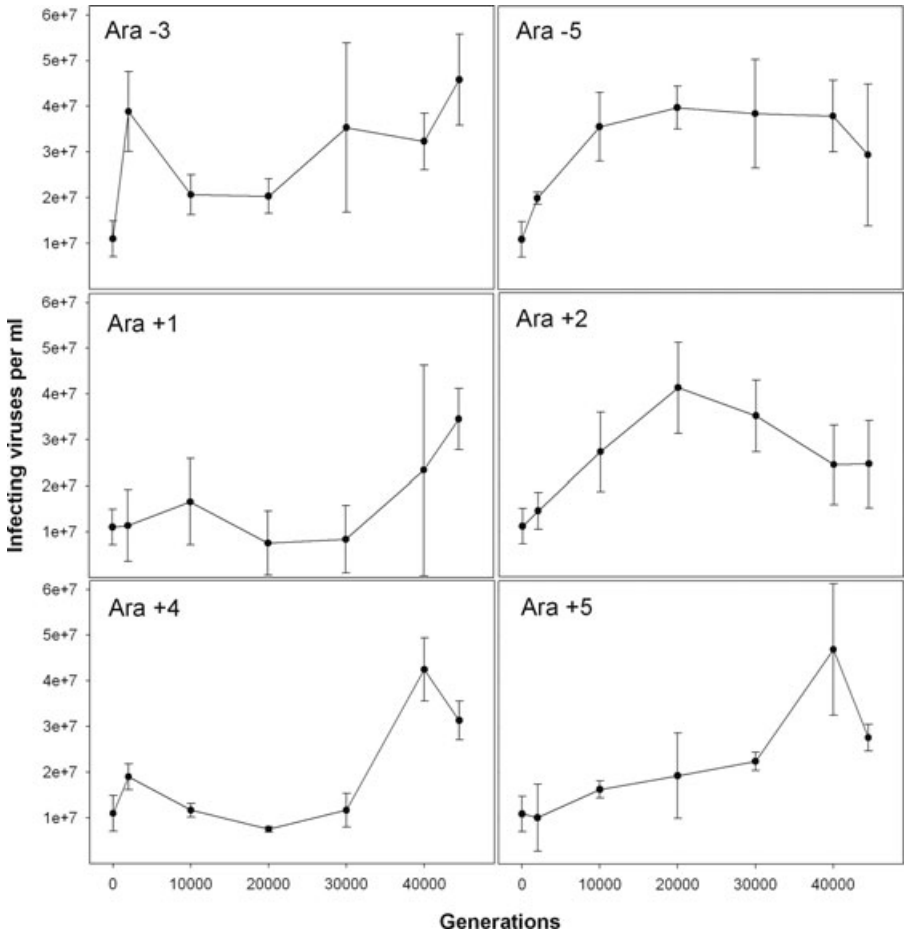

Figure 2. Assays of six evolving *E. coli* populations for susceptibility to infection by phage T6*. Three clones were tested at each time point for each population, except at generation 0, where assays for the same six ancestral clones are shown in all panels. The y-axis shows counts of infecting viruses per milliliter based on plaque-forming units. These counts were all obtained using the same phage stock, and hence the differences across bacterial samples reflect differences in phage infectivity on those bacteria. Each point shows the mean and standard error.

analysis (Table 1), which indicates that the lines showed similar temporal trends (Fig. 1). We note that all lines started from the same ancestral state with respect to phage susceptibility; hence, the significant between-line variation implies some line-by-time interaction, although the statistical analysis was not powerful enough to show it.

In contrast to both the absence of any evolutionary change in resistance to T6 and the evolution of increased susceptibility to T6*, five of the six lines evolved increased resistance to phage λ (Fig. 3). Of those five, four evolved complete resistance, such that no plaques were seen even at the highest phage concentration, and three of them had evolved this resistance by 20,000 generations (Fig. 3). The temporal trend, the heterogeneity among lines, and the line-by-time interaction were all significant in the case of susceptibility to phage λ (Table 1). We sequenced *malT*, a gene that positively regulates the expression of LamB, the surface protein that phage λ targets in susceptible cells, in clones sampled at generation 40,000 from two populations, Ara − 5 and Ara + 5, that evolved complete and partial resistance, respectively. Mutations in *malT* that greatly reduce levels of the LamB protein were previously reported in 20,000-generation clones from populations Ara + 1, Ara + 2, and Ara − 3 (Pelosi et al. 2006), all of which had become completely resistant to λ by that time (Fig. 3). Consistent with the other populations that had evolved complete resistance to phage λ in earlier generations, Ara-5 had a mutation in *malT*. In this case, the change was a C-to-T point mutation at nucleotide position 547, which results in a premature translational stop in the gene (codon 183 out of 902). This mutation likely renders the residual protein nonfunctional and, because the MalT protein is a transcriptional activator of the *lamB* gene, expression of the receptor protein LamB would be eliminated. Population Ara + 5 also acquired a mutation in *malT*, although one with more subtle effects. In particular, it has a C-to-T point mutation at nucleotide position 2570, which changes an alanine to a valine at amino-acid 856. These residues are chemically similar, each being nonpolar, although valine is slightly larger with two more methyl groups.

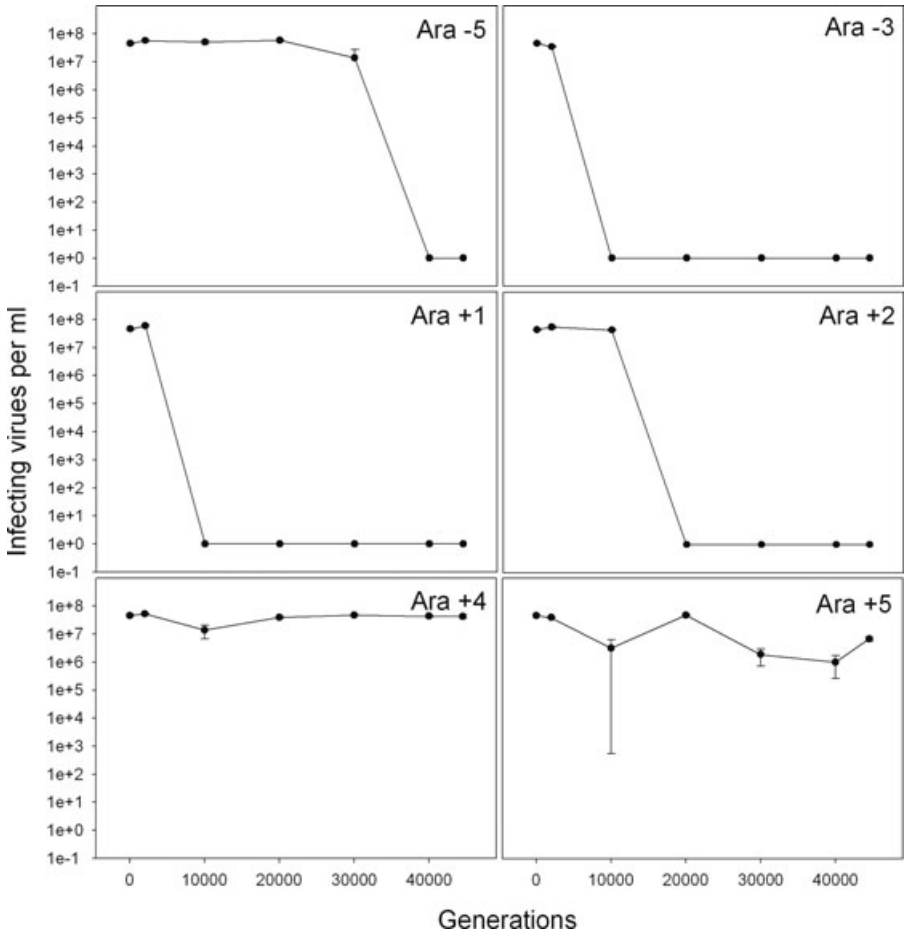

Figure 3. Assays of six evolving *E. coli* populations for susceptibility to infection by phage λ. Three clones were tested at each time point for each population, except at generation 0, where assays for the same six ancestral clones are shown in all panels. Each point shows the mean and standard error. All means were transformed by adding one to plot the zero values on a log scale. y-axis measures the same quantity as defined in Figure 2, but note the difference in scaling. When error bars are not visible, they are smaller than the symbols.

The difference in size evidently affects the structure of the protein, but only slightly, allowing the altered MalT to promote the expression of LamB, although presumably at a reduced level, thereby conferring partial resistance.

Discussion

Resistance to parasites and pathogens often imposes a fitness cost to the host, and such costs have been widely reported for many organisms from bacteria to plants and animals. Resistance functions may be costly either because they are energetically expensive to produce or because they interfere with other cellular or organismal functions. However, some instances of resistance do not incur any measurable fitness costs. For example, resistance by *E. coli* to phage T5 appears to be cost-free under conditions of carbon limitation (Dykhuizen and Hartl 1983; Lenski and Levin 1985); the same mutations are probably deleterious under conditions of iron-limitation, because T5 adsorbs to a receptor protein involved in iron transport (Braun 2009). In a related way, some mutations that confer resistance to phage λ might be neutral, even when the relevant resource is limiting, if the mutations affect the LamB protein's ability to adsorb phage but not its maltodextrin binding and transport functions (Francis et al. 1991). As a eukaryotic example of cost-free resistance, a study of the annual morning glory, *Ipomoea purpurea*, detected no costs of resistance to several insect herbivores (Simms and Rausher 1989), although later work suggested that some costs might have been missed because the resistance traits were not expressed constitutively but instead were inducible (Rausher et al. 1993).

Because so much ecological theory assumes a cost for resistance, it is imperative that more studies are conducted to understand the prevalence of these costs and their long-term effects on the evolution of resistance. Studies extending over many generations have the ability to detect subtle costs that, when accumulated over long periods, may have profound evolutionary effects on resistance traits. In particular, in the prolonged absence of parasites,

host populations are expected to evolve increased sensitivity to the parasites even if there is only a small cost of resistance.

EVOLUTION OF RESISTANCE TO PHAGES T6 AND T6*

We tested this prediction by measuring changes in six *E. coli* populations that initially were resistant to phage T6, but moderately susceptible to a mutant called T6*, across almost 45,000 generations. Convergent increases in sensitivity would lend strong support to the hypothesis of a cost of resistance (i.e., some advantage to becoming susceptible in the absence of viruses), because evolutionary parallelism is a hallmark of adaptation (Simpson 1953; Harvey and Pagel 1991; Travisano et al. 1995; Losos et al. 1998; Crandall et al. 1999; Wichman et al. 1999; Huey et al. 2000; Schluter 2004; Woods et al. 2006).

However, we saw no discernible changes in host susceptibility to T6, as all six evolved lines appeared to be just as resistant as their ancestor. The genetic basis of the ancestor's resistance to T6 was a single point mutation in the *tsx* gene, so there should be little difficulty in evolving lower resistance if that would provide a selective benefit. We sequenced this gene in the six evolved lines, and there were no reversions or any other mutations, fully consistent with the absence of phenotypic changes in susceptibility to T6. We inferred, therefore, that resistance to virus T6 imposes little or no cost to the bacteria under the environmental conditions of the LTEE. This inference was then verified by a direct test that compared the competitive fitness of otherwise isogenic T6-resistant and T6-sensitive strains. This comparison not only found no cost of resistance but, in fact, it revealed an unexpected, albeit slight, competitive advantage to the resistant genotype.

By contrast, all six evolved populations we tested show substantially increased susceptibility to virus T6* (Fig. 1), strongly supporting the hypothesis that resistance to that mutant parasite was costly to the bacteria. However, the physiological basis of that cost is unclear because both the T6* receptor and the genes responsible for the altered resistance are unknown. We detected significant variation among the six lines in their susceptibility to T6* (Table 1), even though the direction of the change was the same in all cases. Although such quantitative variation is less obvious in its phenotypic manifestation than all-or-none resistance, it can have important effects on the ecological and evolutionary dynamics of bacteria–phage interactions (Lenski 1984; Bohannan and Lenski 2000b).

A number of other strikingly convergent changes have previously been reported for populations in the LTEE, including competitive fitness, cell morphology, catabolic niche breadth, and gene expression (Lenski et al. 1991; Lenski and Travisano 1994; Cooper and Lenski 2000; Cooper et al. 2003; Pelosi et al. 2006). These parallel changes have often been imperfect, in the sense of significant among-line variability in the magnitude of the changes (Lenski and Travisano 1994), similar to the heterogeneity in susceptibility to T6* that we observed in this study.

Although such parallel evolutionary changes usually imply adaptation, the precise nature of that adaptation is not always clear. Some parallel changes may reflect selection acting directly on the traits that have been measured, whereas others might instead indicate correlated responses to selection on other traits. It can be difficult to distinguish between these alternatives without precise understanding of the genetic architecture of the relevant traits as well as of the selective forces acting on all the traits. In the case of the LTEE, parallel changes in DNA supercoiling, for example, appear to confer a direct advantage based on genetic manipulations (Crozat et al. 2005). By contrast, losses of physiological functions that are unnecessary in the environment of the LTEE, including some catabolic activities as well as growth capacity at other temperatures, may reflect selection to reduce the costs of unused functions (Cooper and Lenski 2000; Cooper et al. 2001; Sleight et al. 2006).

Without knowing the mechanism of infection by the host range mutant T6*, we cannot distinguish between two opposing hypotheses for the evolution of increased host susceptibility to that mutant phage. On the one hand, selection in the LTEE environment might favor increased production of a cell-surface receptor that provides a direct benefit, such as resource transport. On the other hand, selection might favor reduced production of some metabolically costly molecule that inhibits access by T6* to the receptor it uses for adsorption. However, the lack of subsequent mutations in the *tsx* gene excludes the possibility that increased susceptibility to T6* resulted from a compensatory change in the same gene. Compensatory mutations for costly resistance traits have been reported in studies of bacterial resistance to other viruses and antibiotics (Lenski 1988c; Schrag et al. 1997; Lythgoe and Chao 2003), although compensation often involves mutations in other genes (Moore et al. 2000).

EVOLUTION OF RESISTANCE TO PHAGE λ

In a striking contrast to the evolution of increased susceptibility to T6*, most of the same lines evolved resistance to λ, despite the absence of any phage in the environment of the LTEE. The majority of the lines evolved sudden, qualitative resistance to λ, with that discrete phenotype strongly suggesting that a single mutation might be responsible. This convergent resistance to a parasite that was absent implies that this trait evolved as a pleiotropic side-effect of selection on some other trait, and indeed that is almost certainly the case based on the relationship between our findings and results previously reported in these same lines. In particular, the medium used in the LTEE contains glucose but not maltose, and selection favored mutations that reduce the expression of genes involved in maltose acquisition and catabolism (Pelosi et al. 2006). One of the maltose-related genes that evolved lower

expression encodes the outer membrane protein LamB, which is the receptor that λ exploits to gain entry to the host. Thus, a mutation that was beneficial because of metabolic cost-savings conferred resistance to λ as a pleiotropic effect. In fact, there is a perfect correlation between the lines that we observed to be resistant to λ at generation 20,000 and those with reduced expression of LamB in the same generation (Pelosi et al. 2006).

Furthermore, Pelosi et al. (2006) found the mutations responsible for the lower LamB expression in lines Ara − 3, Ara + 1, and Ara + 2, all of which were in *malT*, a regulatory gene that controls the transcription of *lamB* and other maltose-related genes. These mutations caused deletions and amino-acid substitutions, and the resulting mutant MalT proteins may be unable to bind to the *lamB* promoter sequence (Pelosi et al. 2006). Line Ara − 5 also evolved a similar, discrete resistance phenotype, but after the generation examined by Pelosi et al. (2006). We therefore sequenced clones sampled at 40,000 generations from population Ara − 5 as well as from population Ara + 5, which had evolved low-level quantitative resistance to phage λ infection. The results are strikingly consistent with those of Pelosi et al. (2006). Population Ara − 5 evolved a premature stop early in the *malT* gene that likely destroys its function and, as a consequence, eliminates expression of LamB, consistent with complete resistance to λ in that lineage. Population Ara + 5 had a nonsynonymous mutation in the distal portion of *malT*, one that involves two similar amino acids, consistent with the subtle quantitative resistance in that case.

If such regulatory mutations are beneficial, then why has line Ara + 4 not evolved resistance to phage λ? Given the population sizes and mutation rates in the LTEE, any particular point mutation has likely occurred multiple times in each population (Lenski 2004). One might imagine, therefore, that all beneficial mutations would have had ample opportunity to sweep each population. However, the bacteria in the LTEE are evolving asexually, which leads to competition between co-occurring clones that carry different beneficial mutations, giving rise to the phenomenon of clonal interference (Muller 1932; Gerrish and Lenski 1998). In particular, a beneficial mutation that confers only a small advantage may repeatedly appear, increase in frequency, but be driven extinct before it reaches fixation by other mutations of larger effect. The fitness benefit associated with *malT* mutations has been shown to be significant but small, on the order of 1% (Pelosi et al. 2006), making it among the most subtle beneficial mutations discovered to date in the LTEE. Moreover, one of the three clones we tested from population Ara + 4 at generation 10,000 was completely resistant to phage λ, yet resistant clones were not observed in later generations, indicating that at least one such mutant rose to some intermediate frequency but was eliminated. Given the demonstrated fitness advantage of reducing the expression of the maltose regulon, we anticipate that resistance to phage λ will eventually become fixed in this population as well.

CONCLUSIONS

A substantial body of ecological theory assumes a ubiquitous cost of resistance to parasites, such that resistance should decay when hosts evolve over long periods in the absence of parasites. We saw this dynamic in the case of phage T6*, where all six *E. coli* lines we tested became increasingly susceptible over nearly 45,000 generations in the absence of this parasite. However, we did not see this trend for two other phages. In the case of T6, we saw no changes in host susceptibility in any of those six lines, despite the fact that a single mutation would lead to sensitivity. This outcome suggested that T6-resistance imposes little or no fitness cost on the bacteria in the environment of the LTEE, although it might be costly in some other environments. In fact, we measured a slight advantage for the resistant ancestor over its isogenic sensitive counterpart in direct competition. Even more problematic for the generality of the theory of costly resistance, we observed that most lines we tested evolved increased or complete resistance to phage λ, even though the ancestor was sensitive and none of the lines were exposed to this or any other phage during the LTEE. By combining our observations with other data, we showed that this resistance had evolved as a pleiotropic byproduct of selection on another trait, namely reduced expression of an unused pathway for exploiting a resource that was not present in the experiment. These opposite and unexpected patterns in the evolution of susceptibility to T6* and of resistance to λ illustrate that the genetic and physiological mechanisms that underlie host–parasite interactions can be complex. These complexities may be especially apparent when organisms evolve in changing or novel environments, as in our study. Thus, ecological and evolutionary theories that assume the ubiquity of costs of resistance could lead to incorrect predictions. Whether a cost exists in any particular case may depend on other, seemingly unrelated, aspects of the environment.

When such costs do exist, they will certainly be important for the maintenance of variation in resistance traits in nature. What is sobering, however, is that most studies that attempt to detect trade-offs associated with resistance to natural enemies have been conducted over only one or a few generations, which may be insufficient if the cost is small, standing genetic variation in resistance is absent, or both.

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LITERATURE CITED

Abedon, S. T. 2009. Phage evolution and ecology. Adv. Appl. Microbiol. 67:1–45.

Adams, M. H. 1959. Bacteriophages. Interscience, New York.

Bohannan, B. J. M., and R. E. Lenski. 2000a. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. Ecol. Lett. 3:362–377.

———. 2000b. The relative importance of competition and predation varies with productivity in a model community. Am. Nat. 156:329–340.

Bowen, L., and D. Van Vuren. 1997. Insular endemic plants lack defenses against herbivores. Conserv. Biol. 11:1249–1254.

Braun, V. 2009. FhuA (TonA), the career of a protein. J. Bacteriol. 191:3431–3436.

Bremer, E., A. Middendorf, J. Martinussen, and P. Valentinhansen. 1990. Analysis of the *tsx* gene, which encodes a nucleoside-specific channel-forming protein (Tsx) in the outer-membrane of *Escherichia coli*. Gene 96:59–65.

Brodie, E. D. 1991. Evolutionary response of predators to dangerous prey: reduction of toxicity of newts and resistance of garter snakes in island populations. Evolution 45:221–224.

Buckling, A., Y. Wei, R. C. Massey, M. A. Brockhurst, and M. E. Hochberg. 2006. Antagonistic coevolution with parasites increases the cost of host deleterious mutations. Proc. R. Soc. Lond. B 273:45–49.

Cooper, T. F., D. E. Rozen, and R. E. Lenski. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *E. coli*. Proc. Natl. Acad. Sci. USA 100:1072–1077.

Cooper, V. S., and R. E. Lenski. 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature 407:736–739.

Cooper, V. S., A. F. Bennett, and R. E. Lenski. 2001. Evolution of thermal dependence of growth rate of *Escherichia coli* populations during 20,000 generations in a constant environment. Evolution 55:889–896.

Crandall, K. A., C. R. Kelsey, H. Imamichi, H. C. Lane, and N. P. Salzman. 1999. Parallel evolution of drug resistance in HIV: failure of nonsynonymous/synonymous substitution rate ratio to detect selection. Mol. Biol. Evol. 16:372–382.

Crozat, E., N. Philippe, R. E. Lenski, J. Geiselmann, and D. Schneider. 2005. Long-term experimental evolution in *Escherichia coli*. XII. DNA topology as a key target of selection. Genetics 169:523–532.

Daegelen, P., F. W. Studier, R. E. Lenski, S. Cure, and J. F. Kim. 2009. Tracing ancestors and relatives of *Escherichia coli* B, and the derivation of B strains REL606 and BL21(DE3). J. Mol. Biol. 394:634–643.

Dykhuizen, D. E., and D. L. Hartl. 1983. Selection in chemostats. Microbiol. Rev. 47:150–168.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586–608.

Escobar-Paramo, P., N. Faivre, A. Buckling, C. Gougat-Barbera, and M. E. Hochberg. 2009. Persistence of costly novel genes in the absence of positive selection. J. Evol. Biol. 22:536–543.

Francis, G., L. Brennan, S. Stretton, and T. Ferenci. 1991. Genetic mapping of starch- and Lambda-receptor sites in maltoporin: identification of substitutions causing direct and indirect effects on binding sites by cysteine mutagenesis. Mol. Microbiol. 5:2293–2301.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102/103:127–144.

Gillespie, J. H. 1975. Natural selection for resistance to epidemics. Ecology 65:493–495.

Harvey, P. H., and M. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford.

Herring, C. D., J. D. Glasner, and F. R. Blattner. 2003. Gene replacement without selection: regulated suppression of amber mutations in *Escherichia coli*. Gene 311:153–163.

Huey, R. B., G. W. Gilchrist, M. L. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic decline in size in an introduced fly. Science 287:308–309.

Kato, T., K. Ishida, and H. Sato. 2008. The evolution of nettle resistance to heavy deer browsing. Ecol. Res. 23:339–345.

Lederberg, S. 1966. Genetics of host-controlled restriction and modification of deoxyribonucleic acid in *Escherichia coli*. J. Bacteriol. 91:1029–1036.

Lenski, R. E. 1984. Two-step resistance by *Escherichia coli* B to bacteriophage T2. Genetics 107:1–7.

———. 1988a. Dynamics of interactions between bacteria and virulent bacteriophage. Adv. Microb. Ecol. 10:1–44.

———. 1988b. Experimental studies of pleiotropy and epistasis in *Escherichia coli*: I. Variation in competitive fitness among mutants resistant to virus T4. Evolution 42:425–432.

———. 1988c. Experimental studies of pleiotropy and epistasis in *Escherichia coli*: II. Compensation for maladaptive effects associated with resistance to virus T4. Evolution 42:433–440.

———. 2004. Phenotypic and genomic evolution during a 20,000-generation experiment with the bacterium *Escherichia coli*. Plant Breeding Rev. 24:225–265.

Lenski, R. E., and B. R. Levin. 1985. Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. Am. Nat. 125:585–602.

Lenski, R. E., and M. Travisano. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proc. Natl. Acad. Sci. USA 91:6808–6814.

Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli.* I. Adaptation and divergence during 2,000 generations. Am. Nat. 138:1315–1341.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Loś, J. M., P. Golec, G. Wegrzyn, A. Wegrzyn, and M. Loś. 2008. Simple method for plating *Escherichia coli* bacteriophages forming very small plaques or no plaques under standard conditions. Appl. Environ. Microbiol. 16:5113–5120.

Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodriguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279:2115–2118.

Luria, S. E. 1945. Mutations of bacterial viruses affecting their host range. Genetics 30:84–99.

Lythgoe, K. A., and L. Chao. 2003. Mechanisms of coexistence of a bacteria and a bacteriophage in a spatially homogeneous environment. Ecol. Lett. 6:326–334.

Manning, P. A., and P. Reeves. 1976. Outer membrane of *Escherichia coli* K-12: *tsx* mutants (resistant to bacteriophage T6 and colicin k) lack an outer membrane-protein. Biochem. Biophys. Res. Comm. 71:466–471.

May, R. M., and R. M. Anderson. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Lond. B 219:281–313.

Meyer, J. R., S. P. Ellner, N. G. Hairston, L. E. Jones, and T. Yoshida. 2006. Prey evolution on the time scale of predator-prey dynamics revealed by allele-specific quantitative PCR. Proc. Natl. Acad. Sci. USA 103:10690–10695.

Moore, F. B. G., D. E. Rozen, and R. E. Lenski. 2000. Pervasive compensatory adaptation in *Escherichia coli*. Proc. R. Soc. Lond. B 267:515–522.

Moyed, H. S., T. T. Nguyen, and K. P. Bertrand. 1983. Multicopy Tn*10 tet* plasmids confer sensitivity to induction of *tet* gene expression. J. Bacteriol. 155:549–556.

Muller, H. J. 1932. Some genetic aspects of sex. Am. Nat. 8:118–138.

Pelosi, L., L. Kuhn, D. Guetta, J. Garin, J. Geiselmann, R. E. Lenski, and D. Schneider. 2006. Parallel changes in global protein profiles during long-term experimental evolution in *Escherichia coli*. Genetics 173:1851–1869.

Poullain, V., S. Gandon, M. A. Brockhurst, A. Buckling, and M. E. Hochberg. 2008. The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. Evolution 62:1–11.

Rausher, M. D., K. Iwao, E. L. Simms, N. Ohsaki, and D. Hall. 1993. Induction and the cost of resistance in *Ipomoea purpurea*. Ecology 74:20–29.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning*. Cold Spring Harbor Laboratory Press, Plainview, NY.

Schluter, D. 2004. Parallel evolution and inheritance of quantitative traits. Am. Nat. 163:809–822.

Schneider, H., H. Fsihi, B. Kottwitz, B. Mygind, and E. Bremer. 1993. Identification of a segment of the *Escherichia coli* Tsx protein that functions as a bacteriophage receptor area. J. Bacteriol. 175:2809–2817.

Schrag, S. J., V. Perrot, and B. R. Levin. 1997. Adaptation to the fitness costs of antibiotic resistance in *Escherichia coli*. Proc. Soc. Lond. B 264:1287–1291.

Schwartz, M. 1987. The maltose operon. Pp. 1482–1502 *in* F. C. Neidhardt, ed. *Escherichia coli* and *Salmonella typhimurium*: Cellular and molecular biology. American Society for Microbiology, Washington, DC.

Shonle, I., and J. Bergelson. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). Evolution 54:778–788.

Simms, E. L. 1992. Costs of plant resistance to herbivores. Pp. 392–425 *in* R. S. Fritz, and E. L. Simms, eds. Plant resistance to herbivores and pathogens. Univ. Chicago Press, Chicago.

Simms, E. L., and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. Evolution 43:573–585.

Simpson, G. G. 1953. The major features of evolution. Columbia Univ. Press, New York.

Sleight, S. C, N. S. Wigginton, and R. E. Lenski. 2006. Increased susceptibility to repeated freeze-thaw cycles in *Escherichia coli* following long-term evolution in a benign environment. BMC Evol. Biol. 6:104.

Sleight S. C., C. Orlic, D. Schneider, and R. E. Lenski. 2008. Genetic basis of evolutionary adaptation by *Escherichia coli* to stressful cycles of freezing, thawing and growth. Genetics 180:431–443.

Stahl, E. A., and J. G. Bishop. 2000. Plant-pathogen arms races at the molecular level. Curr. Opin. Plant Biol. 3:299–304.

Stastny, M., U. Schaffner, and E. Elle. 2005. Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? J. Ecol. 93:27–37.

Stowe, K. A. 1998. Experimental evolution of resistance in *Brassica rapa*: correlated response of tolerance in lines selected for glucosinolate content. Evolution 52:703–712.

Studier, F. W., P. Daegelen, R. E. Lenski, S. Maslov, and J. F. Kim. 2009. Understanding the differences between genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3) and comparison of the *E. coli* B and K-12 genomes. J. Mol. Biol. 394:653–680.

Thompson, J. N. 2005. The geographic mosaic of coevolution. Univ. Chicago Press, Chicago.

Travisano, M., J. A. Mongold, A. F. Bennett, and R. E. Lenski. 1995. Experimental tests of the roles of adaptation, chance, and history in evolution. Science 267:87–90.

Van Valen, L. 1973. A new evolutionary law. Evol. Theory 1:1–30.

Wang, Y. F., R. Dutzler, P. J. Rizkallah, J. P. Rosenbusch, and T. Schirmer. 1997. Channel specificity: structural basis for sugar discrimination and differential flux rates in maltoporin. J. Mol. Biol. 272: 56–63.

Wichman, H. A., M. R. Badgett, L. A. Scott, C. M. Boulianne, and J. J. Bull. 1999. Different trajectories of parallel evolution during viral adaptation. Science 285:422–424.

Woods, R., D. Schneider, C. L. Winkworth, M. A. Riley, and R. E. Lenski. 2006. Tests of parallel molecular evolution in a long-term experiment with *Escherichia coli*. Proc. Natl. Acad. Sci. USA 103:9107–9112.

Yoshida, T., N. G. Hairston, and S. P. Ellner. 2004. Evolutionary trade-off between defense against grazing and competitive ability in a simple unicellular alga, *Chlorelia vulgaris*. Proc. R. Soc. Lond. B 271:1947–1953.

Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussmann, and N. G. Hairston. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. Nature 424:303–306.

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Supporting Information

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Figure S1. Percent cells infected, as estimated from the difference in colony-forming bacteria before and after phage T6* was added to liquid cultures.

Table S1. Plaque formation of several phages on several *E. coli* strains.

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