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Repeatability and Contingency in the Evolution of a Key Innovation in Phage Lambda

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Abstract

The processes responsible for the evolution of key innovations, whereby lineages acquire qualitatively new functions that expand their ecological opportunities, remain poorly understood. We examined how a virus, bacteriophage λ , evolved to infect its host, *Escherichia coli*, through a novel pathway. Natural selection promoted the fixation of mutations in the virus's host-recognition protein, J, that improved fitness on the original receptor, LamB, and set the stage for other mutations that allowed infection through a new receptor, OmpF. These viral mutations arose only after the host evolved reduced expression of LamB, whereas certain other host mutations prevented the phage from evolving the new function. This study shows the complex interplay between genomic processes and ecological conditions that favor the emergence of evolutionary innovations.

Throughout the history of life, evolving lineages have acquired qualitatively new functions that enable organisms to expand their ecological opportunities and, in many cases, undergo further diversification (1). Explaining how these transitions have occurred is usually difficult, both because the responsible events typically occurred in the distant past and because their rarity suggests that they might involve atypical evolutionary processes. For example, natural selection is critical for the process of adaptation, yet its role in producing key innovations is less clear because, by fixing variants that improve existing functions, selection might strand populations on local adaptive peaks and thereby prevent them from discovering new functions (2). Darwin was well aware of the difference between improving

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Supporting Online Material

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Materials and Methods

Tables S1–S6

Figs. S1–S7

References (49–74)

an existing trait and evolving a new one (3), and he reasoned that new traits originate by co-opting previously existing structures and functions. Without an understanding of genetic mechanisms, however, he could not provide a detailed account of how this process happens. Since then, others have proposed more explicit models of the origin of new functions that vary in two main respects: the structure of the adaptive landscape – including its dimensionality (2), genetic connectivity (4), and fluctuations caused by changing environments (5) including interactions with coevolving species (6) – and the relative importance of natural selection and random drift (2, 4, 7). However, no consensus has been reached owing, at least in part, to the paucity of cases with sufficient genetic and ecological data (8).

To that end, we examine the evolutionary forces responsible for the emergence of a novel trait in a microbial system, including data bearing on the genetic architecture of the adaptive landscape in which the novel capacity arose. Microbes are well suited for such research because their evolution can be observed in real time, experiments are easily replicated, and transitional states can be studied by reviving samples stored at different times during an experiment (9, 10). We investigated how a virus evolved the ability to infect its host through a new receptor that the ancestral virus cannot use. We tested competing hypotheses about the evolution of this new trait by determining the conditions that promoted its evolution, the mutations that conferred the new function, and the evolutionary forces that drove its emergence.

Study system

Viruses are genetically and morphologically diverse, and they infect all groups of organisms (11, 12). Viruses initiate infections by binding to receptors on the surface of host cells. The physicochemical properties of viral ligands determine which receptors they target and thereby influence the host range and ecological niche of the virus (13). Mutations in viral genes that encode the production of ligands can cause shifts in host range and thus are often associated with emerging diseases (14, 15). The evolution of the ability to infect through a new receptor represents a key innovation for a virus.

The virus we studied, a strictly lytic derivative of phage λ called cI26 (16, table S1), is only known to infect one bacterial species, *Escherichia coli*, and has a specialized ligand, the J protein, at the end of its tail (17). J targets a single protein, LamB, on the *E. coli* outer membrane (17, 18). Phage λ requires only LamB to attach (19), and LamB is the only outer-membrane protein that affects λ reproduction (20).

Given interest in the fundamental question of how organisms evolve novel traits and in the practical problem of how emerging pathogens evolve to target new host receptors, we sought to determine whether λ could evolve to infect through an alternative receptor. Phage λ is well studied and amenable to experimentation (21). A related phage (Ur- λ) possesses side-tail fibers and can infect *E. coli* through a second receptor OmpC (22, 23), suggesting other receptors might be accessible to evolving λ populations. Moreover, we identified conditions that seemed suitable for promoting the use of a novel receptor. In particular, when *E. coli* and λ were cultured together in a glucose-limited environment, the bacteria evolved resistance by mutations in *malt*, which interfered with its role as a transcription factor that promotes *lamB* expression (23). The mutants arose and fixed within 8 days, generating highly resistant populations (fig. S1), although the phage did not go extinct but instead persisted at densities of about 10^6 to 10^7 phage per ml, or about one phage per 10^2 to 10^3 host cells (fig. S2). The phage evidently persisted on a subpopulation of cells that, despite their *malt* mutations, experienced spontaneous induction of the LamB protein (24). This explanation was confirmed by showing that phage were also sustained when they were

grown on a *malT* mutant, whereas the phage went extinct when cultured with a *lamB* mutant that lacks the potential to produce the LamB protein (fig. S3). We reasoned that mutant viruses able to infect through some protein other than LamB would be favored after *malT* mutants arose because they could infect the entire host population rather than a small minority of cells.

Initial evolution experiment

We co-cultured a virulent (non-lysogenic) derivative of λ and *E. coli* B in 10 ml of a minimal glucose medium in six replicate flasks for 28 days with daily transfers of 1% of each community into a flask containing fresh medium, and we preserved samples weekly by freezing 10% of the mixture (16). We tested whether the phage could infect cells through a new receptor by taking samples of the phage populations (typically $\sim 5 \times 10^4$ virions) and inoculating them onto the surface of agar plates infused with a *lamB* mutant that does not produce the LamB receptor. A spot of clearing (lysis of host cells) provided evidence that some phage had evolved the ability to infect through a receptor other than LamB (Fig. 1, panels A–B). Such spots were observed in only one population, but this ability evolved quickly, such that $\sim 0.01\%$ of the phage could infect the *lamB*-negative mutants by day 8, including the isolate designated EvoC, and the majority did so by day 15.

Identification of the novel receptor

We used seven knockout strains (derivatives of K12 BW25113), each missing a gene encoding a different outer-membrane protein, to identify the new receptor (16, 25). We then introduced *malT* mutations to these strains so that they also would not express the native LamB receptor. We inferred the new receptor by testing the ancestral and evolved λ against these double mutants to see which ones were resistant to the various phage isolates. The only host that was resistant to the EvoC isolate was the *ompF malT* double mutant that lacked expression of both OmpF and LamB (Fig. 1, table S2), indicating that OmpF was the new receptor. This evolved phage could still infect the host strain expressing LamB but not OmpF, indicating that the phage retained the ability to infect through its native receptor (Fig. 1).

Both LamB and OmpF form trimeric porins composed of three identical β barrels (26, 27). This overall structure is probably essential for the J protein in the λ tail to bind because J, too, forms a trimer and is thought to attach with radial symmetry across the three pore domains (28). Although OmpF has the most similar crystal structure to LamB of any *E. coli* protein determined to date (29), they are not the most similar pair by amino-acid sequence (table S3). This discordance suggests that the overall structure is at least as important for λ binding as the identity of specific amino-acid residues. Also, OmpF is the sole major porin in the *E. coli* B strain used in this study, and B expresses it constitutively during growth (30, 31). Hence, OmpF provided a substantial ecological opportunity to phage that evolved the ability to target it.

Genome evolution

We sequenced the genome of the evolved phage EvoC in order to identify the mutations that allowed it to use the OmpF receptor (16). There were five mutations in total, and all of them were in the J gene (Fig. 2). Targeted sequencing of J showed that a single substitution (A to G at position 3034) differentiated EvoC from another evolved isolate from the same time point, EvoA, that could use only the ancestral LamB receptor, indicating that mutation contributed to the new receptor function. Another LamB-dependent phage from the same day, EvoB, differed from EvoC at five sites in the J protein.

Large-scale evolution experiment

We repeated our first experiment with 96 more communities to identify general principles of how λ evolves the capacity to target an alternative receptor (16). We sampled daily for finer resolution of the evolutionary dynamics. As before, only some phage populations (24/96) evolved the ability to use a second receptor. This ability emerged about the same time (median 12 days; range 9–17 days; fig. S4), and all isolates with altered receptor function infected hosts through the OmpF protein.

Parallel molecular evolution

We sequenced J alleles from 24 phage isolates that independently evolved the ability to target OmpF during the large-scale evolution experiment to determine whether the mutation at position 3034 or any others were required to use that receptor. The isolates were taken the same day the new function was detected (16). For comparison, we sequenced phage from 24 populations that never evolved this trait; these isolates were sampled on the same days as those that evolved the new trait, so that the elapsed times were the same.

In total, there were 241 single nucleotide polymorphisms (SNPs) across the 48 J alleles, but no insertions or deletions (Fig. 3). However, there were only 40 unique mutations because many arose repeatedly in replicate populations. Moreover, all of them were non-synonymous. The alleles for phage able to target OmpF had on average 6.63 (± 0.51 95% confidence interval (CI)) SNPs, whereas the phage that required LamB had only 3.42 (± 0.50 95% CI) SNPs. This difference is highly significant based on a paired comparison between the two types of phage matched for the day of their isolation and, in the case of multiple equivalent pairs, matched arbitrarily by position in the experiment ($t_s = 9.144$, 23 d.f., two-tailed $p < 0.0001$; table S4). Also, across both classes of phage, over 97% of the mutations were in the last 25% of the protein (C-terminal end), the region known to interact with LamB (32).

There are four striking cases of parallel evolution of the J protein in the phage that target OmpF. In two cases, the mutations were identical across all 24 populations, while in two others there were slight variations (Fig. 3). In particular, all J alleles from phage able to infect through OmpF had the A-to-G mutation at nucleotide position 3034 and G-to-A mutation at position 3319. Also, all of them had a mutation at either position 3320 or 3321, affecting the same codon (amino-acid residue 1107) as the mutation at position 3319. Finally, all J alleles had at least one mutation between positions 2969 and 2999 (amino-acid residues 990 to 1000).

Each of these mutations or classes of mutation was also found in at least one of the phage that retained the ancestral host-range, although none of them had all four together (Fig. 3). Two LamB-dependent isolates, F2 and H4, had three of the mutations, as did EvoA from the initial experiment (Fig. 2), yet none produced clearing on lawns of *lamB* mutants.

The correspondence between the use of the OmpF receptor and the presence of these four mutations, coupled with the observation that phage having only three of the four cannot use OmpF, provides evidence that all four are required for λ to infect through OmpF. We performed two additional assays to confirm that only phage with all four mutations can infect *lamB* mutants (16). The assays were performed using isolates EvoA, F2, and H4 that each had three of the four canonical mutations and D7 that had all four and no others. Only D7 exhibited a measureable adsorption rate on *lamB* mutant cells (fig. S5), and it was also the only one that reproduced on *lamB* mutants in the medium used in the evolution experiments (fig. S6). These findings indicate an “all-or-none” form of epistasis among the four mutations responsible for the novel receptor phenotype.

Role of natural selection

In the λ population that evolved to use OmpF in the initial experiment, the A3034G mutation was the fourth and final step, and it was clearly advantageous because it conferred the ability to infect the entire cell population. However, the all-or-none epistasis among the mutations means that selection for that new capacity *per se* was not responsible for the rise of the three prior mutations. Nonetheless, there are several lines of evidence that selection drove their rise. First, all 248 independent mutations in the 51 sequenced J alleles were non-synonymous, whereas the expected ratio of non-synonymous to synonymous changes is 3.19:1 under the null model for the ancestral J sequence (16). This great excess is evident even if we include only the 82 non-synonymous mutations in the 24 isolates that did not evolve the new receptor function. Second, the mutations are highly concentrated in the region of the J protein that interacts directly with LamB (18). Third, there was parallel evolution at the genetic level across the populations. For those phage that evolved to exploit OmpF, an average of 61% (4.06/6.63) of mutations were shared across independently derived pairs (fig. S7), which greatly exceeds the fraction expected under a conservative randomization test (16) using only the variable sites in J ($p < 10^{-5}$). Pairs of phage that remained dependent on LamB shared on average 17% (0.58/3.42) of their mutations (fig. S7), and this fraction is again significant under the same test ($p < 10^{-5}$). Thus, it is clear that selection acted on the J protein even before the new capacity evolved. This selection presumably improved the interaction of the phage tail fibers with LamB.

Stochasticity and contingency

All of the λ populations had the same ecological incentive to exploit an alternative receptor, but only some evolved that ability. Why were some populations successful and others not? One possibility is that all of them would eventually have evolved that function, but there was insufficient time to do so. This explanation is consistent with the facts that the LamB-dependent isolates had fewer mutations than those able to target OmpF, and that the two groups shared many mutations. Alternatively, the evolution of the new receptor function might have been contingent on earlier events (33–35), such that particular changes in the phage or the host promoted or impeded the subsequent evolution of that function. To test these hypotheses, we replayed evolution (35, 36) using various combinations of phage and bacteria.

First, we tested whether certain mutations in the phage that might enhance performance on LamB would impede the evolution of the new OmpF function. We inoculated flasks with the ancestral bacteria and one of six phage isolates. Three of the six phage isolates had different sets of three mutations that were present in multiple isolates that evolved to use the OmpF receptor, including one, zero, and two of the four canonical mutations. The other three isolates had three, two, and one mutations that were not observed in any isolate that previously evolved the ability to use OmpF; these isolates also each had one of the canonical mutations. The first set provided a positive control; the second set had candidate mutations for impeding the evolution of the new function. For each phage, we propagated 12 communities for 10 days and surveyed daily the phage's ability to lyse *lamB* mutant cells. There were as many or more successes in evolving the new function among the three phages that had the potentially interfering mutations as among the positive controls (Fig. 4A). This experiment thus provides no evidence that some phage failed to evolve the new function because they had mutations that prevented them from doing so.

Next, we asked if the outcome was contingent on mutations in the evolving bacteria. To that end, we performed a similar replay experiment except that the initial phage type was held constant while the starting bacterial isolate was varied. For the phage, we used EvoA, an

isolate that was one mutation away from using OmpF (Fig. 2). For the bacteria, we used six clones: three from communities where λ evolved to use OmpF, and three where the phage retained their dependence on LamB. We observed a striking “all-or-none” pattern of outcomes, though not in accordance with our categories (Fig. 4B). In particular, all 36 λ populations evolved the final mutation required to use OmpF in communities with three bacterial clones whereas none of the phage evolved that ability in 36 communities with the three other clones. For two of the latter class (EcA8 and EcF6), the phage were unable to reproduce and went extinct; in the other case (EcC3), phage persisted but none of the replays yielded phage able to use OmpF. It is clear that bacterial characteristics determined whether the phage would evolve the new receptor function. However, the bacteria that promoted that outcome did not necessarily come from communities in which λ had previously evolved to exploit OmpF.

We sequenced the full genomes of these six bacteria to identify the mutations responsible for the differences in phage evolution. The six genomes harbor a total of 15 mutations (table S5). Five have similar deletions that impact the *rbs* operon, which confers the ability to grow on ribose; previous work has shown that these deletions occur at an unusually high rate owing to a nearby insertion-sequence element in the ancestral strain (37). All 10 other mutations are directly related to the interaction with λ . As expected, all six genomes have mutations in *malT* that confer resistance to the ancestral phage, as described earlier. One genome from a community where λ evolved to use the OmpF receptor has a non-synonymous mutation in the *ompF* gene, which might confer partial resistance to the evolved phage. The three remaining mutations disrupt *manY* or *manZ*, and they uniquely differentiate the three strains that prevented phage from evolving to use OmpF from the three strains that allowed that change (table S5). The *manY* and *manZ* genes encode the transmembrane channel of the ManXYZ mannose permease, which is required for λ DNA to cross the inner membrane (38–40). These mutations thus confer resistance by blocking a later step during infection, and they would render ineffective any phage mutations that altered the receptor function. Therefore, the evolution of phage that target OmpF is promoted by bacterial mutations in *malT* but impeded by mutations in *manYZ*, indicating contingency dependent on the host-parasite coevolution.

After discovering the *manYZ* mutations, we screened all 96 bacterial populations from the large-scale experiment to determine how many harbored mannose-deficient mutants that would block λ infections (16). At least 80 populations (table S6) had such mutations, including many from communities in which λ evolved the ability to exploit the OmpF receptor. However, these mutations rarely fixed; instead, susceptible subpopulations persisted in 77 of the 80 communities that allowed the phage to continue to evolve. This finding suggests a complex interplay between coevolving phage and bacteria, one that depends on the entire community and its diversity. To test this hypothesis, we repeated the second replay experiment using the same phage and bacteria except with bacterial communities instead of clones (16). Once again, some bacteria consistently impeded the evolution of phage that used OmpF while others consistently promoted that change (Fig. 4C). Moreover, those outcomes differed for one clone (EcA8, Fig. 4B) and its community (ComA8, Fig. 4C), confirming the effect of bacterial diversity on the phage’s evolution.

Repeatability, contingency, and the evolution of a key innovation

Phage λ often, but not always, evolved the ability to infect its *E. coli* hosts by targeting a new receptor, OmpF. Figure 5 summarizes the important steps in this process, including some that promoted this key innovation and others that impeded it. The fact that several mutations are required for λ to use OmpF may explain why no previous studies have reported this change, despite decades of intense study of this phage. However, by running

evolution experiments rather than mutational screens, we observed 25 cases in which this new function evolved. Our experiments are not the first to demonstrate evolutionary transitions in viruses (13, 41–43); one study found that a single mutation allowed phage $\phi 6$ to infect a new host species (13), while a study of phage SBW25 Φ 2 showed that coevolution experiments were more effective at producing host-range shifts than were screens using new hosts (43). The rich and complex dynamics of coevolving species may thus sometimes facilitate key innovations (6, 44).

More generally, our study shows some of the challenges that make it difficult to observe and explain the origin of many new functions including the requirement for multiple mutations, the complex interactions of mutations within and between species, and the resulting historical contingency that can enable or impede the outcome of interest depending on the order in which mutations occur. The “all-or-none” epistasis among the four canonical phage mutations implies that it would have been unlikely for the new function to evolve on the scale of our experiments, except for the lucky fact that some of the mutations were beneficial to the phage in performing their current function, thereby pushing evolution toward the new function. The mutations in the bacteria, and how they influenced the phage’s evolution, were also important. In particular, the initial resistance mutation generated a physiological subpopulation of hosts that allowed the phage to persist and adapt to the original receptor, thereby accumulating the required mutations. Yet, as fortuitous as these circumstances were, another mutation could – and often did – derail the emergence of the new function: namely, a mutation that conferred an alternative mode of host resistance eliminated the advantage to the phage of targeting the OmpF receptor. The interactions between bacteria and phage, which contributed so much to the development of microbial genetics and molecular biology (45, 46), continue to serve as powerful models to study ecology and evolution (47, 48).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Mayr, E. *Animal Species and Evolution*. Belknap Press; Cambridge, MA: 1963.
2. Gavrillets, S. *Toward an Extended Evolutionary Synthesis*. Pigliucci, M.; Muller, G., editors. MIT Press; Cambridge, MA: 2010.
3. Darwin, C. *The Origin of Species*. Murray; London: 1859.
4. Fisher, RA. *The Genetical Theory of Natural Selection*. Clarendon Press; Oxford: 1930.
5. Dieckmann, U.; Doebeli, M.; Metz, JAJ., editors. *Adaptive Speciation*. Cambridge Univ. Press; Cambridge, UK: 2004.
6. Thompson, JN. *The Geographic Mosaic of Coevolution*. Univ. Chicago Press; Chicago: 2005.

7. Wright S. *Genetics*. 1931; 16:97. [PubMed: 17246615]
8. Pigliucci M. *Philos Sci*. 2008; 75:887.
9. Dennehy JJ. *Trends Microbiol*. 2009; 17:450. [PubMed: 19765997]
10. Elena SF, Lenski RE. *Nat Rev Genet*. 2003; 4:457. [PubMed: 12776215]
11. Edwards RA, Rohwer F. *Nat Rev Microbiol*. 2005; 3:504. [PubMed: 15886693]
12. Torsvik V, Ovreas L, Thingstad TF. *Science*. 2002; 296:1064. [PubMed: 12004116]
13. Duffy S, Burch CL, Turner PE. *Evolution*. 2007; 61:2614. [PubMed: 17908251]
14. Pepin KM, Lass S, Pulliam JRC, Read AF, Lloyd-Smith JO. *Nat Rev Microbiol*. 2010; 8:802. [PubMed: 20938453]
15. Holmes EC. *Annu Rev Ecol Evol Syst*. 2009; 40:353.
16. Materials and methods are available as supporting material on *Science* Online.
17. Katsura, I. Lambda II. Hendrix, RW., editor. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1981.
18. Berkane E, et al. *Biochem*. 2006; 45:2708. [PubMed: 16489764]
19. Grayson P, Han L, Winther T, Phillips R. *Proc Natl Acad Sci USA*. 2007; 104:14652. [PubMed: 17804798]
20. Maynard ND, et al. *PLoS Genet*. 2010; 6:e1001017. [PubMed: 20628568]
21. Hendrix, RW., editor. Lambda II. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1983.
22. Hendrix RW, Duda RL. *Science*. 1992; 258:1145. [PubMed: 1439823]
23. Gallet R, Kannoly S, Wang I. *BMC Microbiol*. 2011; 11:181. [PubMed: 21827665]
24. Braunbreton C, Hofnung M. *Mol Gen Genet*. 1978; 159:143. [PubMed: 345088]
25. Baba T, et al. *Mol Syst Biol*. 2006; 2:2006.0008 .
26. Schirmer T, Keller TA, Wang YF, Rosenbusch JP. *Science*. 1995; 267:512. [PubMed: 7824948]
27. Kefala G, et al. *Protein Sci*. 2010; 19:1117. [PubMed: 20196071]
28. Gurnev PA, Oppenheim AB, Winterhalter M, Bezrukov SM. *J Mol Biol*. 2006; 359:1447. [PubMed: 16697410]
29. Koebnik R, Locher KP, Van Gelder P. *Mol Microbiol*. 2000; 37:239. [PubMed: 10931321]
30. Schneider D, et al. *BMC Microbiol*. 2002; 2:18. [PubMed: 12106505]
31. Crozat E, et al. *J Bacteriol*. 2011; 193:429. [PubMed: 21097626]
32. Wang J, Hofnung M, Charbit A. *J Bacteriol*. 2000; 182:508. [PubMed: 10629200]
33. Gould, SJ. *Wonderful Life*. Norton; New York: 1989.
34. Travisano M, Mongold JA, Bennett AF, Lenski RE. *Science*. 1995; 267:87. [PubMed: 7809610]
35. Blount ZD, Borland CZ, Lenski RE. *Proc Natl Acad Sci USA*. 2008; 105:7899. [PubMed: 18524956]
36. Woods RJ, et al. *Science*. 2011; 331:1433. [PubMed: 21415350]
37. Cooper VS, Schneider D, Blot M, Lenski RE. *J Bacteriol*. 2001; 183:2834. [PubMed: 11292803]
38. Erni B, Zanolari B, Kocher HP. *J Biol Chem*. 1987; 262:5238. [PubMed: 2951378]
39. Saris PEJ, Palva ET. *FEMS Microbiol Lett*. 1987; 44:371.
40. Esquinas-Rychen M, Erni B. *J Mol Microbiol Biotechnol*. 2001; 3:361. [PubMed: 11361066]
41. Graham RL, Baric RS. *J Virol*. 2010; 84:3134. [PubMed: 19906932]
42. Ferris MT, Joyce P, Burch CL. *Genetics*. 2007; 176:1013. [PubMed: 17409090]
43. Hall AR, Scanlan PD, Buckling A. *Am Nat*. 2011; 177:44. [PubMed: 21117957]
44. Thompson, JN. *The Coevolutionary Process*. Univ. Chicago Press; Chicago, IL: 1994.
45. Friedman DI, Court DL. *Curr Opin Microbiol*. 2001; 4:201. [PubMed: 11282477]
46. Cairns, J.; Stent, GS.; Watson, JD., editors. *Phage and the Origins of Molecular Biology*. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2007.
47. Bohannan BJM, Lenski RE. *Ecol Lett*. 2000; 3:362.
48. Flores C, Meyer JR, Valverde S, Farr L, Weitz JS. *Proc Natl Acad Sci USA*. 2011; 108:E288. [PubMed: 21709225]

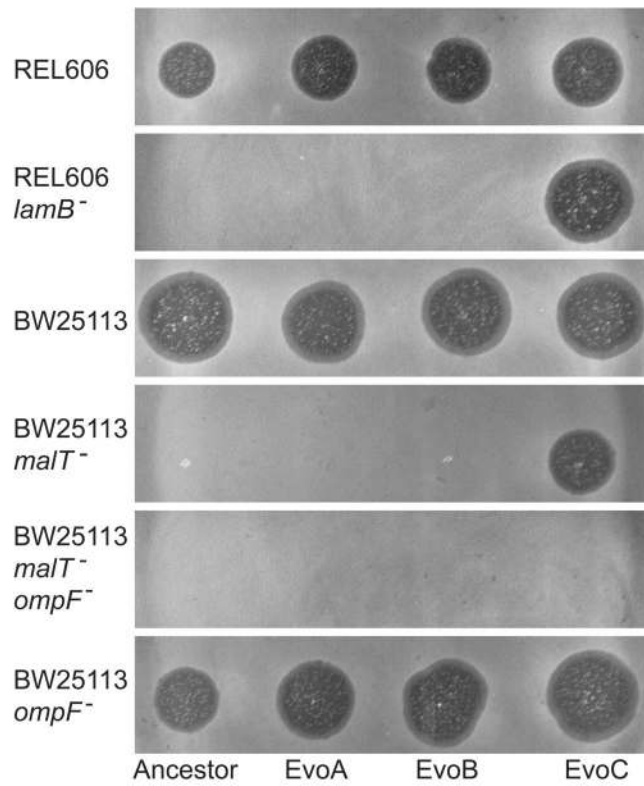


Fig. 1.

Infection assay for four λ isolates tested on six *E. coli* strains. Each panel shows a bacterial lawn with aliquots of the phage applied to it; darker regions indicate successful infections that clear the lawn. The phage isolates include the ancestor and three clones isolated from the same population on day 8 of the initial evolution experiment including one, EvoC, that can use the OmpF receptor. The bacterial strains include mutants that differ in the expression of LamB and OmpF porins on two genomic backgrounds, REL606 (the ancestral strain in the evolution experiments) and BW25113 (a derivative of K12). The *malt*⁻ strains do not express LamB at appreciable levels.

	C2988A	C2999T	A3034G	T3230C	G3319A	T3321A	A3364T
EvoA							
EvoB							
EvoC							

Fig. 2. Mutations in the λ gene encoding the J protein in three isolates from the same population on day 8 of the initial evolution experiment. The isolates are shown in rows and the mutations in columns, with the first letter being the ancestral nucleotide, the number the nucleotide position, and the last letter the evolved nucleotide. The gray fill indicates that the phage isolate has the corresponding mutation.

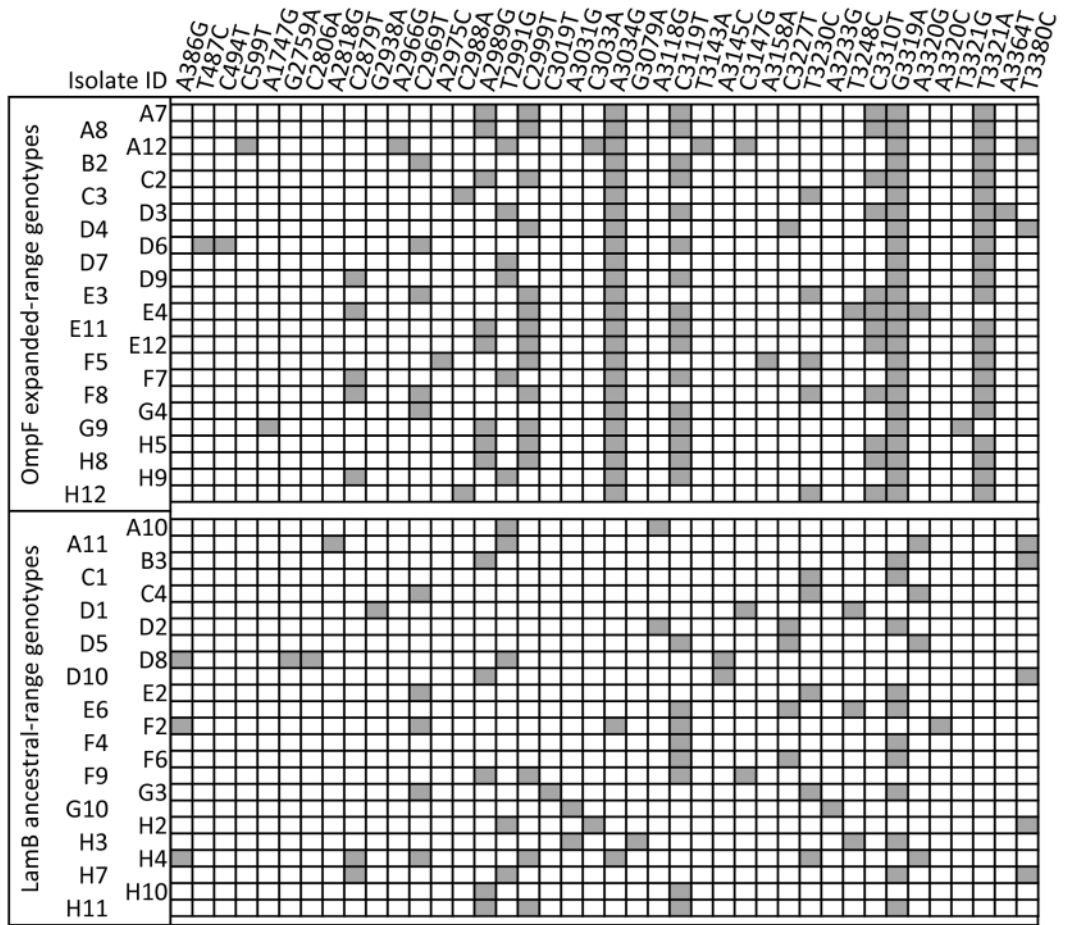
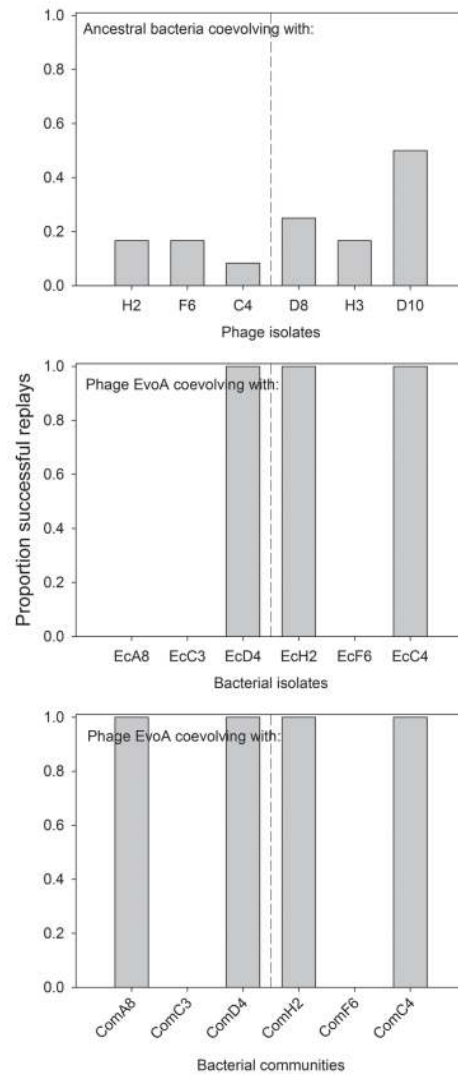


Fig. 3. Mutations affecting the J protein in phage isolates from 48 independent populations of the large-scale experiment. Isolates are shown in rows (with alternate labels offset for readability) and mutations in columns; gray fill indicates an isolate has the mutation. The top 24 rows show phage isolates that can target the new OmpF receptor; the bottom 24 rows show phage that remain dependent on Lamb.

**Fig. 4.**

Replay experiments using different combinations of phage and bacteria. For each panel, the y-axis shows the proportion of replicate replays that produced phage able to target the new OmpF receptor. Top panel: Replays were initiated with the ancestral bacteria and six phage isolates. Each combination was replicated 12-fold. Three of the phage (H2, F6, and C4) had mutations shared by multiple lineages that evolved the capacity to target OmpF in the large-scale experiment. The other three (D8, H3, and D10) had mutations that were never observed in phage that targeted OmpF. The latter mutations were candidates for impeding the evolution of the new function, but that hypothesis was not supported. Middle panel: Replays were initiated with phage EvoA (which needs only one more mutation to use OmpF) and six bacterial clones. Each combination was replicated 4-fold. Three clones (EcA8, EcC3, and EcD4) were isolated from flasks in which phage evolved the capacity to target OmpF in the large-scale experiment. The other three (EcH2, EcF6, and EcC4) came from flasks in which phage did not evolve that function. The replay outcomes did not support these categories, but sequencing the bacterial genomes identified mutations that uniquely determined whether the phage would evolve the OmpF function. See text for details. Bottom panel: Replays were initiated using the same phage and bacteria used in the middle panel, except with full bacterial communities rather than individual clones. Each

combination was replicated 12-fold. The different outcomes for one bacterial clone (EcA8, middle) and its source community (ComA8, bottom) show the effect of bacterial diversity on phage evolution.

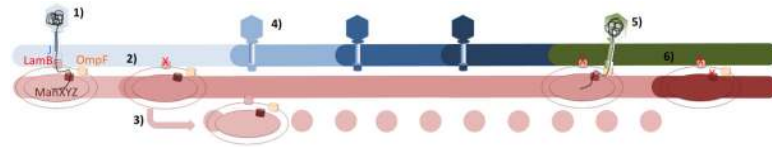


Fig. 5.

Steps in the coevolution of phage λ and its *E. coli* host leading to the phage's ability to target a new receptor, OmpF. (1) The ancestral phage targets the LamB porin using the J protein and injects its DNA into the periplasm, then the DNA is transported into the cytoplasm via the ManXYZ permease. (2) The bacteria evolve resistance by mutations in *maltT*, a positive regulator of LamB expression. (3) However, spontaneous inductions of LamB generate a subpopulation of phenotypically sensitive cells that can sustain the phage population. (4) The phage evolves mutations in the J protein that improve performance on the LamB receptor. Some of these mutations are also required for the phage to target OmpF. (5) The phage eventually evolves the four mutations that enable it to use OmpF. (6) However, the bacteria may evolve additional resistance by mutations in *manY* or *manZ* that prevent transport of the phage DNA into the cytoplasm. When these mutants become sufficiently common, there is little or no benefit to mutant phage that can use OmpF.