Sustained coevolution of phage Lambda and *Escherichia coli* involves inner as well as outer membrane defenses and counter-defenses

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**Running Head:** Bacteria-phage coevolution

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Abstract

Bacteria often evolve resistance to phage through the loss or modification of cell-surface receptors. In *Escherichia coli* and phage λ, such resistance can catalyze a coevolutionary arms race focused on host and phage structures that interact at the outer membrane. Here, we analyze another facet of this arms race involving interactions at the inner membrane, whereby *E. coli* evolves mutations in mannose permease-encoding genes *manY* and *manZ* that impair λ’s ability to eject its DNA into the cytoplasm. We show that these *man* mutants arose concurrently with the arms race at the outer membrane. We tested the hypothesis that λ evolved an additional counter-defense that allowed them to infect bacteria with deleted *man* genes. The deletions severely impaired the ancestral λ, but some evolved phage grew well on the deletion mutants, indicating they regained infectivity by evolving the ability to infect hosts independently of the mannose permease. This coevolutionary arms race fulfills the model of an inverse-gene-for-gene infection network. Taken together, the interactions at both the outer and inner membranes reveal that coevolutionary arms races can be richer and more complex than is often appreciated.

IMPACT STATEMENT

Laboratory studies of coevolution help us understand how host defenses and pathogen counter-defenses change over time, which is often essential for predicting the future dynamics of host-pathogen interactions. One particular model, termed “inverse-gene-for-gene” coevolution, predicts that coevolution proceeds through alternating steps, whereby hosts lose the features exploited by pathogens, and pathogens evolve to exploit alternative features. Using a classic model system in molecular biology, we describe the
nature and timing of a previously overlooked step in the coevolution of *E. coli* and bacteriophage lambda. Our work demonstrates that this mode of coevolution can profoundly re-shape the interactions between bacteria and phage.

**INTRODUCTION**

An issue of longstanding interest is whether the coevolution of bacteria and virulent (lytic) phages involves endless rounds of bacterial defenses and phage counter-defenses. Based on experiments in chemostats, Lenski and Levin (1) suggested that bacteria typically had the upper hand, as *Escherichia coli* often eventually evolved resistance by deleting or inactivating the phage’s specific receptor, which the phage could not readily overcome. This resistance did not imply the extinction of the phage, however, because it often reduced the bacteria’s competitiveness for resources. Instead, the typical outcome was coexistence of resistant and sensitive bacteria, with the latter more efficient at exploiting resources and thus able to sustain the phage’s persistence (2, 3). A study of cyanobacteria and their phages in the marine environment also supported this pattern (4).

On the other hand, Lenski and Levin also pointed out that bacteria would lose the upper hand if the phage targeted a receptor that was essential for the bacteria to survive in their current environment. They cited then-recent work by Williams Smith & Huggins (5, 6), who showed they could successfully treat mice with otherwise lethal bacterial infections using a phage that specifically targeted a receptor required for the bacteria to colonize the mice. As the problem of bacterial resistance to antibiotics has grown, similar strategies are now being tested in which phage that specifically target drug-efflux pumps are deployed as therapeutic agents (7-9). In the meantime, yet other forms of bacteria-
Phage coevolution have been discovered, including CRISPR systems in bacteria and countermeasures to avoid these defenses in phage (10-13).

Another part of the argument that bacteria had the upper hand in the coevolutionary arms race depended on the idea that, while phages could often counter minor mutations in receptors, it was much more difficult for them to evolve the ability to use another receptor if the bacteria simply stopped producing the usual receptor (1). However, more recent work has shown that some host-phage pairs can undergo longer coevolutionary cycles involving defenses and counter-defenses at the outer membrane (14-16), and some phages can evolve to use new receptors even on a short time scale (17). This coevolutionary dynamic – in which hosts lose structures exploited by specific pathogens, and those pathogens evolve to exploit alternative structures – is called inverse-gene-for-gene (IGFG) coevolution (18-21). This IGFG framework is useful for representing changes in coevolving communities of bacteria and phage (Fig. 1). For example, if phage cannot evolve to exploit new features after bacteria have evolved resistance, then phage populations may be evolutionarily static (22, 23). Conversely, if phage exploit essential features of the bacteria that cannot be eliminated, then the host’s evolution is constrained and phage infectivity may remain elevated (6, 8). Our study builds on one such example of IGFG coevolution, in which it was discovered that populations of a virulent strain of phage λ often evolved the ability to use another outer-membrane receptor after coevolving E. coli reduced their expression of the receptor that the phage had initially exploited (17, 24).

Phage λ requires a two-step infection process to cross the outer and inner bacterial membranes (Fig. S1). The λ tail initiates infection at the outer membrane of the cell,
where its J protein fibers adsorb to the bacterial protein LamB (25, 26). The tail proteins V and H allow λ to enter the periplasm and thereby interact with the mannose permease proteins (encoded by manY and manZ) in the inner membrane, which λ uses to eject its genome into the cytoplasm (27-30). Resistance to λ can occur by blocking λ’s entry at either the outer or inner membrane, with resistance mutations typically mapping to lamB, lamB’s positive regulator malT (25, 26), or the mannose permease genes (27, 28, 30) (Fig. S1). It has been shown that sensitive E. coli and lytic λ can coexist, along with resistant E. coli mutants, in both continuous (31) and batch culture regimes (17). Previous analysis of this coevolving system has revealed IGFG dynamics focused on outer membrane defenses and counter-defenses. That is, E. coli often first evolves malT mutations that reduce LamB expression, resulting in increased resistance to λ (17, 31, 32), and λ then regains infectivity through mutations in the J gene that increase its adsorption rate and fitness (31, 33). In some, but not all, experiments, specific sets of J mutations allow the novel exploitation of a second outer membrane protein, OmpF, catalyzing further evolution including mutations in the ompF gene (17, 34).

Despite extensive knowledge about the evolution of the initial (adsorption) and final (lysis) steps of λ infection of E. coli, much less is known about the evolution of the genetic networks during other stages of infection, including λ’s passage through the periplasmic space and the ejection of its DNA into the host cytoplasm. Meyer et al. (17) found that E. coli coevolving with λ often acquired mutations that impacted their ability to grow on mannose, which presumably were favored because they disrupt entry of the phage genome via the mannose permease. In this study, we examine how this coevolutionary arms race – previously focused on the cell’s outer membrane – also set off
an arms race involving the host’s inner membrane, including the mechanism λ uses to
eject its DNA through that membrane and into the bacteria’s cytoplasm.

**METHODS**

**Bacteria and phage strains**

Meyer *et al.* (17) founded 96 replicate cultures with *E. coli* B strain REL606 and lytic
phage λ cI26, serially passaged the communities for 20 days, and froze mixed-
community samples daily. Some of the phage populations evolved the ability to use the
outer membrane protein OmpF as a receptor, some of the bacterial populations evolved
mutations that affected mannose metabolism, and some communities changed in both
respects. We obtained phage isolates from two of the populations (Table 1, Pop-A and
Pop-B) that changed in both of these key respects; in each case, however, the isolates
were taken four days before the phage had evolved the new ability to use the OmpF
receptor (Table 1, Supplementary Material). *E. coli* K12 strains BW25113, JW1807, and
JW1808 are from the Keio collection (35). REL606 ΔmanZ was constructed using a two-
step allelic exchange (Supplementary Material, Table S1, and Table S2).

**Phage growth assays**

We measured the population growth of the ancestral and evolved phages under the same
culture conditions as those in which the communities evolved (17) (Supplementary
Material). The initial densities were ~9 × 10⁶ cells per ml and ~1 × 10⁴ phage per ml. We
calculate the phage’s net population growth as the ratio of its final density after one day
to its initial density; we show the resulting net growth on a log₈0-transformed scale. We
enumerated the initial and final phage populations using dilution plating and soft-agar
overlays (Supplementary Material). We performed 5 or 6 replicate assays for each phage-host combination shown in Figure 2.

**Frequency of mutants with altered mannose phenotypes**

We estimated the frequency of bacteria with mutations affecting the mannose permease by plating from the time-series of frozen samples taken from populations Pop-A and Pop-B on tetrazolium mannose agar, as done previously (17). Mutants with reduced ability to metabolize mannose form deeply pigmented colonies that can be readily distinguished from those of the ancestral strain REL606, which forms light pink colonies on that medium.

*Data accessibility:* Data are available as Supplementary Datasets S1 (net population growth of phage λ on wild type and knockout bacteria) and S2 (temporal dynamics of *man* mutants in *E. coli* populations).

**RESULTS AND DISCUSSION**

Our experiments focus on two independently coevolved communities of mixed *E. coli* and λ populations, designated Pop-A and Pop-B (17). Both λ populations evolved from a common ancestral phage (strain cI26). From each evolved population, we isolated a single phage clone: λ-A from Pop-A and λ-B from Pop-B (Table 1). Each clone was isolated 4 days before its population evolved the ability to use the OmpF receptor; hence, the phage clones were isolated on different days of the coevolution experiment performed by Meyer et al. (17).

To examine whether and how coevolution affected λ’s dependence on the ManY and ManZ proteins, we measured the population growth of the ancestral (cI26) and the two
coevolved phage isolates (λ-A and λ-B) on bacterial strains with and without the manY and manZ genes (Table 1). Both the ancestral and evolved phage isolates grew well on bacterial strains with intact manY and manZ genes, including both the ancestral E. coli B strain, REL606, used in the coevolution experiment, and the K12 genetic background in which the Keio collection was made (Fig. 2, Table 1). Deletion of either the manY or manZ gene in either background severely reduced the ancestral phage’s population growth. In two cases (REL606 ∆manZ and Keio ∆manY), we saw no growth whatsoever in the ancestral phage (cI26) population after 24 hours; in the other case (Keio ∆manZ), the ancestral phage population increased ~10-fold, but that was five orders of magnitude less than the increase on the same background with both mannose permease genes present. In striking contrast, both evolved phage isolates showed substantial growth on all three bacterial strains that lacked either the manY or manZ gene (Fig. 2). These results thus indicate an inverse-gene-for-gene coevolutionary interaction at the inner membrane. That is, the bacteria modified or lost the mannose permease, which the ancestral phage used to eject its genome into the cytoplasm, and the phage countered by evolving independence of that function.

To determine when the mutant mannose permease mutants arose in the two E. coli populations studied here, we plated frozen samples from the coevolution experiments on tetrazolium mannose agar, on which man mutants form pigmented colonies distinguishable from the wild type (Supplementary Material) (36). We are particularly interested in the timing of the appearance of the man mutants relative to two other steps in the coevolutionary arms race that were previously characterized: (i) the malT mutations that reduced the bacteria’s expression of LamB and thus the adsorption of the
ancestral phage (33); and (ii) λ’s new ability to adsorb to OmpF as an alternative receptor (17). Our phage-growth data demonstrate that manY and manZ deletions confer substantial resistance even to the ancestral phage, which can use only the LamB surface receptor (Fig. 2). That result suggests the possibility that the man mutants could have arisen early in the coevolution experiments, perhaps alongside or even before the malT mutations that provided resistance at the outer membrane. However, time-course data show that the man alleles consistently reached high frequencies (above the detection limits, shown as gray dashed lines in Fig. 3) only after the fixation of the malT mutations, which occurred by day 8 in both populations studied here (17) (Fig. 3, Fig. 4, Table S3).

These temporal data also show that the man mutations had nearly fixed in both bacterial populations (frequencies >95% on day 10 in Pop-A and on day 12 in Pop-B), but then the mutants sharply declined the next day. This reversal suggests these mutants were killed by phages that evolved independence of the mannose permease, and it is consistent with previous data showing that mutant man alleles rarely fixed in the bacterial populations (17). Meyer et al. (Fig. S2 in (17)) reported that the bacterial population densities remained high (∼2 × 10^9 cells per ml, near the carrying capacity of the medium) throughout this period of the evolution experiment. Therefore, the mutant frequencies that we observed (Fig. 3) correspond to ∼4 × 10^7 cells per ml (about 2% of the total population, the limit of detection in that assay) to almost 2 × 10^9 cells per ml (the carrying capacity). With such large population sizes, any phage mutants that gained the ability to infect the man mutants would have access to a large number of hosts, and correspondingly, a large fitness benefit. The resulting growth of the man-independent phage population would drive the frequency of man mutants down, especially if the man-
independent phages preferentially infected and killed the man mutants relative to other
cells that retained the wild-type permease. Fitness costs associated with loss of the
mannose permease may also have contributed to the reversal, although the costs of the
resistance mutations are small compared to their benefit in the presence of phage (36).

In host Pop-A, variation in colony morphology further suggested that different man
alleles were present before and after the sudden decline in the frequency of man mutants
on day 11 (Fig. 4, Supplementary Material). The initial boom and bust of the mutant man
alleles in both populations also occurred before the phage had evolved to use OmpF (Fig.
3, dashed arrows). Whether λ gained independence from the mannose permease by
exploiting another inner membrane protein, and whether E. coli did (or could) respond by
eliminating such a structure, are interesting questions for future work.

Our results are broadly consistent with genetic and molecular biology studies of λ
host-range mutations. Scandella and Arber (30) isolated E. coli mutants that allowed
phage adsorption to the cell envelope but interfered with ejection of the phage genome,
thereby reducing infection success to a small fraction of that observed on wild-type cells.
The responsible mutations were mapped to the mannose permease operon (27, 37), and λ
mutants that could infect these mutant bacteria had mutations in phage genes V or H (38).
Mutations in V and H have also been observed in another population in this study system
(39). Williams et al. (37) found that, for E. coli strain K12, manZ is not strictly required
for wild-type λ to eject its genome, and our results accord with that finding (Fig. 2, Keio
background). However, our results suggest that λ cI26 does require manZ when infecting
E. coli strain B, at least in the culture conditions that we used (Fig. 2, REL606
background). Alternatively, λ cI26 might occasionally infect and replicate in hosts
without \textit{manZ}, but at a rate that is offset by the decay or inactivation of free virus particles under these conditions (17, 33). In any case, the net population growth of the ancestral phage on either the \textit{ΔmanY} or \textit{ΔmanZ} bacteria is insufficient to offset the 100-fold daily dilutions (Fig. 2, dashed line) that took place during the coevolution experiment (17).

Taken together, our results imply that \textit{E. coli} and \textlambda coevolved in an inverse gene-for-gene manner (18) (Fig. 1). This coevolution involved two infection steps – crossing first the outer and then the inner membrane – and at least three, and probably four, distinct host features (Figs. 1, 5, and S1). \textit{E. coli} evolved resistance to phage \textlambda through the loss or alteration of maltose transport across the outer membrane (via mutations in \textit{malT}) and mannose transport across the inner membrane (via mutations in \textit{manY} or \textit{manZ}), while \textlambda evolved to exploit other \textit{E. coli} features including another outer membrane protein (OmpF) and, presumably, some as yet unidentified, alternative inner membrane protein (shown as encoded by the hypothetical \textit{inx} gene in Fig. 5). While our study addresses one particular bacteria-phage interaction in a simple laboratory setting, it illustrates the extent to which the resulting coevolutionary arms races can be richer and more complex than is often appreciated.

There are many alternative coevolutionary paths through an inverse-gene-for-gene network that has four features subject to host defenses and parasite counter-defenses (Fig. 5). This multiplicity of potential paths suggests that mutation and selection could drive replicate communities to different regions of the coevolutionary landscape, raising other interesting questions. How might different first-step resistance mutations affect the subsequent host-range evolution of the phage and the further evolution of host resistance?
To what extent can IGFG systems continuously evolve host defenses and parasite
counter-defenses? What is the effect of such prolonged coevolution for community
diversity? Do communities become increasingly divergent as the coevolving populations
follow different paths through the network, or might they eventually converge on the
same phenotypic states after a period of divergence? How important are evolutionary
innovations in opening new paths, relative to pleiotropic tradeoffs that may close off
certain paths? Future work should investigate these and other questions about the
coevolution of bacteria and phage and the structure of their genetic interaction networks.

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AUTHOR CONTRIBUTIONS
A.R.B., R.M.S., and R.E.L. conceived the study. A.R.B., R.M.S., and J.G. performed the
experiments. All authors analyzed the data and wrote the manuscript.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

REFERENCES
1. Lenski RE, Levin BR. 1985. Constraints on the coevolution of bacteria and virulent
phage: a model, some experiments, and predictions for natural communities. The
American Naturalist 125:585-602.
2. Chao L, Levin BR, Stewart FM. 1977. A complex community in a simple habitat: an
experimental study with bacteria and phage. Ecology 58:369-378.
3. Levin BR, Stewart FM, Chao L. 1977. Resource-limited growth, competition, and
predation: a model and experimental studies with bacteria and bacteriophage. The
American Naturalist 111:3-24.
4. Waterbury JB, Valois FW. 1993. Resistance to co-occurring phages enables marine
Synechococcus communities to coexist with cyanophages abundant in seawater. Applied
and environmental microbiology 59:3393-3399.
5. Smith HW, Huggins MB. 1980. The association of the O18, K1 and H7 antigens and the
CoIV plasmid of a strain of Escherichia coli with its virulence and immunogenicity.
Microbiology 121:387-400.
6. Smith HW, Huggins MB. 1982. Successful treatment of experimental Escherichia coli
infections in mice using phage: its general superiority over antibiotics. Journal of General
Microbiology 128:307-318.
7. Burmeister AR, Fortier A, Roush C, Lessing AJ, Bender RG, Barahman R, Grant
R, Chan BK, Turner PE. 2020. Pleiotropy complicates a trade-off between phage
resistance and antibiotic resistance. Proc Natl Acad Sci U S A 117:11207-11216.
8. Chan BK, Sistrom M, Wertz JE, Kortright KE, Narayan D, Turner PE. 2016. Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. Sci Rep 6:26717.

9. Kortright KE, Chan BK, Koff JL, Turner PE. 2019. Phage therapy: A renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe 25:219-232.

10. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science 315:1709.

11. Shmakov S, Smargon A, Scott D, Cox D, Pyzocha N, Yan W, Abudayyeh OO, Gootenberg JS, Makarova KS, Wolf YI, Severinov K, Zhang F, Koonin EV. 2017. Diversity and evolution of class 2 CRISPR–Cas systems. Nature Reviews Microbiology 15:169-182.

12. Westra ER, Levin BR. 2020. It is unclear how important CRISPR-Cas systems are for protecting natural populations of bacteria against infections by mobile genetic elements. Proceedings of the National Academy of Sciences 117:27777-27785.

13. Pawluk A, Davidson AR, Maxwell KL. 2018. Anti-CRISPR: discovery, mechanism and function. Nature Reviews Microbiology 16:12+.

14. Buckling A, Rainey PB. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. Proceedings of the Royal Society of London Series B: Biological Sciences 269:931-936.

15. Scanlan PD, Buckling A. 2012. Co-evolution with lytic phage selects for the mucoid phenotype of Pseudomonas fluorescens SBW25. The ISME journal 6:1148-1158.

16. Scanlan PD, Hall AR, Lopez-Pascua LDC, Buckling A. 2011. Genetic basis of infectivity evolution in a bacteriophage. Molecular Ecology 20:981-989.

17. Meyer JR, Dobias DT, Weitz JS, Barrick JE, Quick RT, Lenski RE. 2012. Repeatability and contingency in the evolution of a key innovation in phage lambda. Science 335:428-432.

18. Fenton A, Antonovics J, Brockhurst MA. 2009. Inverse-gene-for-gene infection genetics and coevolutionary dynamics. Am Nat 174:E230-242.

19. Fenton A, Antonovics J, Brockhurst MA. 2012. Two-step infection processes can lead to coevolution between functionally independent infection and resistance pathways. Evolution 66:2030-2041.

20. Sieber M, Robb M, Forde SE, Gudelj I. 2014. Dispersal network structure and infection mechanism shape diversity in a coevolutionary bacteria-phage system. ISME Journal 8:504-514.

21. Agrawal A, Lively CM. 2002. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. Evolutionary Ecology Research 4:79-90.
22. Dennehy JJ. 2012. What can phages tell us about host-pathogen coevolution? Int J Evol Biol 2012:396165.

23. Koskella B, Brockhurst MA. 2014. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS Microbiol Rev 38:916-931.

24. Chaudhry WN, Pleška M, Shah NN, Weiss H, McCall IC, Meyer JR, Gupta A, Guet CC, Levin BR. 2018. Leaky resistance and the conditions for the existence of lytic bacteriophage. PLOS Biology 16:e2005971.

25. Hofnung M, Jezierska A, Braun-Breton C. 1976. lamB mutations in E. coli K12: growth of lambda host range mutants and effect of nonsense suppressors. Mol Gen Genet 145:207-213.

26. Thirion JP, Hofnung M. 1972. On some genetic aspects of phage lambda resistance in E. coli K12. Genetics 71:207-216.

27. Elliott J, Arber W. 1978. E. coli K-12 pel mutants, which block phage lambda DNA injection, coincide with ptsM, which determines a component of a sugar transport system. Mol Gen Genet 161:1-8.

28. Erni B, Zanolari B, Kocher HP. 1987. The mannose permease of Escherichia coli consists of three different proteins: amino acid sequence and function in sugar transport, sugar phosphorylation, and penetration of phage lambda DNA. J Biol Chem 262:5238-5247.

29. Esquinas-Rychen M, Erni B. 2001. Facilitation of bacteriophage lambda DNA injection by inner membrane proteins of the bacterial phosphoenol-pyruvate: carbohydrate phosphotransferase system (PTS). J Mol Microbiol Biotechnol 3:361-370.

30. Scandella D, Arber W. 1974. An Escherichia coli mutant which inhibits the injection of phage lambda DNA. Virology 58:504-513.

31. Spanakis E, Horne MT. 1987. Co-adaptation of Escherichia coli and coliphage λvir in continuous culture. J Gen Microbiol 133:353-360.

32. Meyer JR, Agrawal AA, Quick RT, Dobias DT, Schneider D, Lenski RE. 2010. Parallel changes in host resistance to viral infection during 45,000 generations of relaxed selection. Evolution 64:3024-3034.

33. Burmeister AR, Lenski RE, Meyer JR. 2016. Host coevolution alters the adaptive landscape of a virus. Proc Roy Soc B: Biol Sci 283:20161528.

34. Meyer JR, Flores CO, Weitz JS, Lenski RE. 2008. Key innovation in a virus catalyzes a coevolutionary arms race. ALife Proceedings 13:532-533.

35. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol 2:2006 0008.
36. **Burmeister AR, Sullivan R, Lenski RE.** 2020. Fitness costs and benefits of resistance to phage lambda in experimentally evolved *Escherichia coli*, p 123-143. *In* Banzhaf W, Cheng B, Deb K, Holekamp K, Lenski RE, Ofria C, Pennock R, Punch B, Whittaker D (ed), Evolution in action: past, present, and future. Springer, New York, NY.

37. **Williams N, Fox DK, Shea C, Roseman S.** 1986. Pel, the protein that permits lambda DNA penetration of *Escherichia coli*, is encoded by a gene in *ptsM* and is required for mannose utilization by the phosphotransferase system. *Proc Natl Acad Sci U S A* 83:8934-8938.

38. **Scandella D, Arber W.** 1976. Phage lambda-DNA injection into *Escherichia coli pel* mutants is restored by mutations in phage gene V or gene H. *Virology* 69:206-215.

39. **Gupta A, Peng S, Leung CY, Borin JM, Weitz JS, Meyer JR.** 2020. Leapfrog dynamics in phage-bacteria coevolution revealed by joint analysis of cross-infection phenotypes and whole genome sequencing. *bioRxiv* doi:10.1101/2020.10.31.337758:2020.2010.2031.337758.
Figures

A  Gene-for-gene coevolution:
Host evolves by gaining resistance genes, and pathogen evolves by losing genes that elicit host defense.

“Gain of function”
resistance evolution
(e.g., ability to detect flagella)

“Loss of function”
virulence evolution
(e.g., loss of flagella)

B  Inverse gene-for-gene coevolution:
Host evolves resistance by losing structures, and pathogen evolves to exploit other structures.

“Loss of function”
(e.g., loss of LamB and maltose transport)

“Gain of function”
(e.g., ability to infect through OmpF)

Fig. 1. Genetic interaction networks during gene-for-gene (GFG) coevolution (panel A) and inverse-gene-for-gene (IGFG) coevolution (panel B). In both scenarios, host alleles affect selection on pathogen phenotypes, and pathogen alleles influence selection on host phenotypes. However, the two models have different implications for understanding historical coevolution and predicting
future changes. During GFG coevolution, hosts evolve resistance by gaining resistance genes, and pathogens evolve by losing genes that elicit host defenses. GFG coevolution is common among plants and their bacterial pathogens; it may also occur in bacteria-phage interactions that involve restriction-modification and CRISPR defenses. During IGFG coevolution, pathogen infectivity requires the exploitation of specific host features, and resistance involves eliminating the exploited features. Unlike in the GFG model, host defenses in the IGFG model do not require pathogen recognition, and the pathogen’s evasion of host resistance does not require the loss of a defense elicitor.
**Fig. 2.** Net population growth of phage $\lambda$ on wild type, $\Delta$manY, and $\Delta$manZ bacteria. Whether the phage could grow was assessed by performing one-tailed $t$-tests on the log$_{10}$-transformed ratio of phage population densities at the start and end of a one-day cycle, with the null hypothesis of zero growth ($**$, $p < 0.001$; **, $0.001 < p < 0.01$; ns, not significant, $p > 0.05$). Each test was based on 5 or 6 replicate assays. Phage isolates $\lambda$-A and $\lambda$-B evolved in a batch-culture regime with 100-fold dilution each day, and so 100-fold growth was required for their persistence; this break-even level is indicated by the dashed line.
Fig. 3. Temporal dynamics of man mutants in E. coli populations Pop-A (panel A) and Pop-B (panel B). Mutant malT alleles had already reached fixation in both populations by day 8 (17). Bacteria with man mutations, which confer resistance to the ancestral phage λ, rose to high frequencies and then declined sharply in abundance in both populations after day 8, but before λ had evolved to use the alternative receptor OmpF (timing indicated by vertical dashed arrows). These data imply that the man mutations evolved on malT mutant backgrounds, and that λ evolved independence of the mannose permease – causing the
precipitous decline in the frequency of \textit{man} mutants – before it evolved the ability
to use OmpF. The shaded regions indicate the maximum and minimum
frequencies of the \textit{man} mutants based on analyzing two samples per population
each day (mean $N = 90$ colonies tested per sample, minimum 29 colonies). The
horizontal gray dashed lines show the approximate limit of detection of the \textit{man}
mutants (0.019 for panel A, 0.022 for panel B).
**Fig. 4.** Evolution of *man*-related colony morphology on tetrazolium mannose agar. *E. coli* mutants with reduced ability to metabolize mannose form more deeply pigmented colonies than the wild type bacteria. Three representative colonies are shown for each sample from days 1-20 of two coevolution experiments. Representative colonies within a column are from the same agar plate and shown at the same magnification after incubation for 18-21 hours. Panel A: Pop-A. Panel B: Pop-B. Panel C: Comparison of wild type and Δ*manZ* bacteria in the same *E. coli* strain B genetic background.
Fig. 5. An inverse-gene-for-gene model showing the structure of the genetic network for coevolving *E. coli* and *λ* populations. Columns indicate bacterial genotypes with four exploitable features, and rows indicate *λ* genotypes that exploit those features: *mal*, maltose transport across the outer membrane; *man*, mannose transport across the inner membrane; *ompF*, glucose and electrolyte transport across the outer membrane; *imx*, a hypothetical inner membrane feature that is exploited by *λ* that evolved independence of the mannose permease. The "+" symbol indicates that either the bacteria have the feature or the phage exploit the feature. The "−" symbol indicates the bacteria lack the feature, express it to a reduced degree, or otherwise modify it to minimize phage infection. Asterisks (*) indicate infectivity for each host-phage pair, with more asterisks indicating greater infectivity. Adaptive changes through the network can
proceed by two types of moves: *E. coli* resistance (to the right across rows), and increased λ infectivity (downward across columns). The coevolving communities were founded by host genotype a and phage genotype vi (shown by the black circle). The communities analyzed in this study appear to have moved through the shaded nodes in five steps, as indicated by the arrows.
### Table 1. *E. coli* and phage λ strains used in this study.

| Bacteria Clones: | Description | Relevant Characteristics |
|------------------|-------------|--------------------------|
| REL606           | *E. coli* B ancestor of coevolution experiment | malT*, ompF*, manY*, manZ* |
| REL606 Δ*manZ*   | *manZ* deletion, derived from REL606<sup>a</sup> | Δ*manZ* |
| BW25113          | *E. coli* K12 parental strain of Keio collection | malT*, ompF*, manY*, manZ* |
| JW1807           | *manY* deletion in Keio collection | Δ*manY* |
| JW1808           | *manZ* deletion in Keio collection | Δ*manZ* |
| DH5α             | Strain used for λ plaque-based enumeration | malT*, ompF*, manY*, manZ* |

| Phage Clones: | Description | Relevant Characteristics |
|---------------|-------------|--------------------------|
| cI26          | Lytic λ ancestor of both phage populations | Requires *E. coli* LamB |
| λ-A           | Evolved λ isolate from Pop-A<sup>b</sup> on Day 8 (4 days before the population evolved to use OmpF) | Requires *E. coli* LamB |
| λ-B           | Evolved λ isolate from Pop-B<sup>b</sup> on Day 11 (4 days before the population evolved to use OmpF) | Requires *E. coli* LamB |

<sup>a</sup>This strain also has three mutations that have no known relevance to interactions with phage λ (Supplementary Material). For construction methods, see Supplementary Material, Table S1, and Table S2.

<sup>b</sup>For simplicity, we have designated the source populations Pop-A and Pop-B. These correspond to population numbers D9 and G9 in the original experiment described by Meyer et al. (17).