

human islets. Additionally, rational programming of designer cells enables (iv) straightforward fine-tuning of performance parameters and provides (v) flexibility to couple glucose sensing to the production of other therapeutic proteins such as GLP-1 required for T2D therapy (1, 29).

REFERENCES AND NOTES

- S. Cornell, *Ther. Clin. Risk Manag.* **11**, 621–632 (2015).
- J. Tuomilehto, S. Bahjri, *Nat. Rev. Endocrinol.* **12**, 127–128 (2016).
- K. S. Polonsky, *N. Engl. J. Med.* **367**, 1332–1340 (2012).
- S. J. Cleland, *Nat. Rev. Endocrinol.* **8**, 476–485 (2012).
- G. I. Shulman, *N. Engl. J. Med.* **371**, 1131–1141 (2014).
- S. M. Grundy, *Nat. Rev. Drug Discov.* **5**, 295–309 (2006).
- P. M. Rao, D. M. Kelly, T. H. Jones, *Nat. Rev. Endocrinol.* **9**, 479–493 (2013).
- J. E. Bruin *et al.*, *Stem Cell Rep.* **4**, 605–620 (2015).
- A. Rezaei *et al.*, *Nat. Biotechnol.* **32**, 1121–1133 (2014).
- J. C. Pickup, *N. Engl. J. Med.* **366**, 1616–1624 (2012).
- D. J. Drucker, M. A. Nauck, *Lancet* **368**, 1696–1705 (2006).
- G. M. Church, M. B. Elowitz, C. D. Smolke, C. A. Voigt, R. Weiss, *Nat. Rev. Mol. Cell Biol.* **15**, 289–294 (2014).
- F. W. Pagliuca *et al.*, *Cell* **159**, 428–439 (2014).
- B. E. Tuch *et al.*, *Gene Ther.* **10**, 490–503 (2003).
- C. Aguayo-Mazzucato, S. Bonner-Weir, *Nat. Rev. Endocrinol.* **6**, 139–148 (2010).
- L. Marzban *et al.*, *Diabetes* **55**, 2192–2201 (2006).
- S. A. Stanley *et al.*, *Science* **336**, 604–608 (2012).
- H. Ye, M. Daoud-EI Baba, R. W. Peng, M. Fussenegger, *Science* **332**, 1565–1568 (2011).
- F. M. Ashcroft, P. Rorsman, *Nat. Rev. Endocrinol.* **9**, 660–669 (2013).
- M. D'Arco, A. C. Dolphin, *Sci. Signal.* **5**, pe34 (2012).
- D. Ausländer *et al.*, *Mol. Cell* **55**, 397–408 (2014).
- H. Ye *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 141–146 (2013).
- J. W. Rooney, M. R. Hodge, P. G. McCaffrey, A. Rao, L. H. Glimcher, *EMBO J.* **13**, 625–633 (1994).
- M. Elsnér, M. Tiedge, B. Guildbakke, R. Munday, S. Lenzen, *Diabetologia* **45**, 1542–1549 (2002).
- R. B. Robey, N. Hay, *Oncogene* **25**, 4683–4696 (2006).
- H. J. Jang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 15069–15074 (2007).
- F. Macian, *Nat. Rev. Immunol.* **5**, 472–484 (2005).
- A. J. Vegas *et al.*, *Nat. Med.* **22**, 306–311 (2016).
- G. G. Holz 4th, W. M. Kühtreiber, J. F. Habener, *Nature* **361**, 362–365 (1993).
- S. J. Russell *et al.*, *N. Engl. J. Med.* **371**, 313–325 (2014).
- T. J. Kieffer, *Cell Stem Cell* **18**, 699–702 (2016).
- J. T. McCluskey *et al.*, *J. Biol. Chem.* **286**, 21982–21992 (2011).
- D. W. Scharp, P. Marchetti, *Adv. Drug Deliv. Rev.* **67**, 68, 35–73 (2014).
- P. Saxena *et al.*, *Nat. Commun.* **7**, 11247 (2016).
- A. N. Zaykov, J. P. Mayer, R. D. DiMarchi, *Nat. Rev. Drug Discov.* **15**, 425–439 (2016).
- M. Kastellorizos, N. Tzipnis, D. J. Burgess, *Adv. Exp. Med. Biol.* **865**, 93–108 (2015).
- F. M. Wurm, *Nat. Biotechnol.* **22**, 1393–1398 (2004).

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SUPPLEMENTARY MATERIALS

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VIRAL EVOLUTION

Ecological speciation of bacteriophage lambda in allopatry and sympatry

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Understanding the conditions that allow speciation to occur is difficult because most research has focused on either long-lived organisms or asexual microorganisms. We propagated bacteriophage λ , a virus with rapid generations and frequent recombination, on two *Escherichia coli* host genotypes that expressed either the LamB or OmpF receptor. When supplied with either single host (allopatry), phage λ improved its binding to the available receptor while losing its ability to use the alternative. When evolving on both hosts together (sympatry), the viruses split into two lineages with divergent receptor preferences. Although the level of divergence varied among replicates, some lineages evolved reproductive isolation via genetic incompatibilities. This outcome indicates that, under suitable conditions, allopatric and sympatric speciation can occur with similar ease.

Studies of extant and extinct species provide evidence that biological evolution can promote diversification rather than mere replacement of one form by another (1). Moreover, asexual microbes frequently diversify when cultured in the laboratory (2), even in simple conditions that would seem to favor homogeneity (3). Sympatric diversification in sexual populations has long been thought to be difficult because constant recombination prevents an interbreeding population from splitting into genetically distinct lineages that occupy different ecological niches (4, 5). However, putative examples of sympatric speciation have advanced the argument that speciation in a recombining population may occur under certain circumstances (6, 7), although other studies have disputed these claims (8). This debate is important because the process of speciation is fundamental to biological evolution.

To shed light on this question, we conducted evolution experiments with viruses to examine the effect of recombination on their divergence into distinct ecological niches. We studied a virulent (i.e., strictly lytic) derivative of phage λ , which infects *Escherichia coli*. Most λ can use only a single outer-membrane protein, LamB, as a receptor, but the strain we studied, EvoC, can also exploit a second receptor, OmpF (9). The ability

to use OmpF arose via five point mutations in the host-recognition gene, *J*. To examine λ diversification, we propagated virus populations on one or both of two strains of *E. coli* that differ only in whether they possess the *lamB* or *ompF* genes, which encode LamB and OmpF, respectively (10). Phages tend to exploit specific receptors (11), leading us to predict that populations of the generalist phage EvoC would evolve toward increased receptor specificity. Whether or not two receptor specialists evolved, however, would likely also depend on the effects of recombination. When two or more λ viruses infect the same cell, their genomes can recombine (12).

We use λ as a model for analyzing the mechanisms of speciation. Viruses are diverse (13), and their rates and mechanisms of speciation will depend on their life histories, just as the rates and mechanisms vary in plants and animals. However, some salient features of speciation are shared, even across such distant taxa. For example, viruses that infect the same species and cell types are thought to have evolved mechanisms to limit recombination, including divergences in nucleotide composition and RNA structure that are analogous to prezygotic barriers in plants and animals (14–16). One feature of our system that might promote sympatric speciation is the connection between the viruses' ecological niches (i.e., host cells) and where genetic exchange occurs. Specialists that use the same receptor will naturally tend to recombine because they are more likely to infect the same host. Therefore, reproductive isolation can evolve as a by-product of ecological divergence, without requiring other mutations that govern recombination. Such dual-effect mutations have been described in other systems and are important because their simultaneous effects on ecological niches and mating

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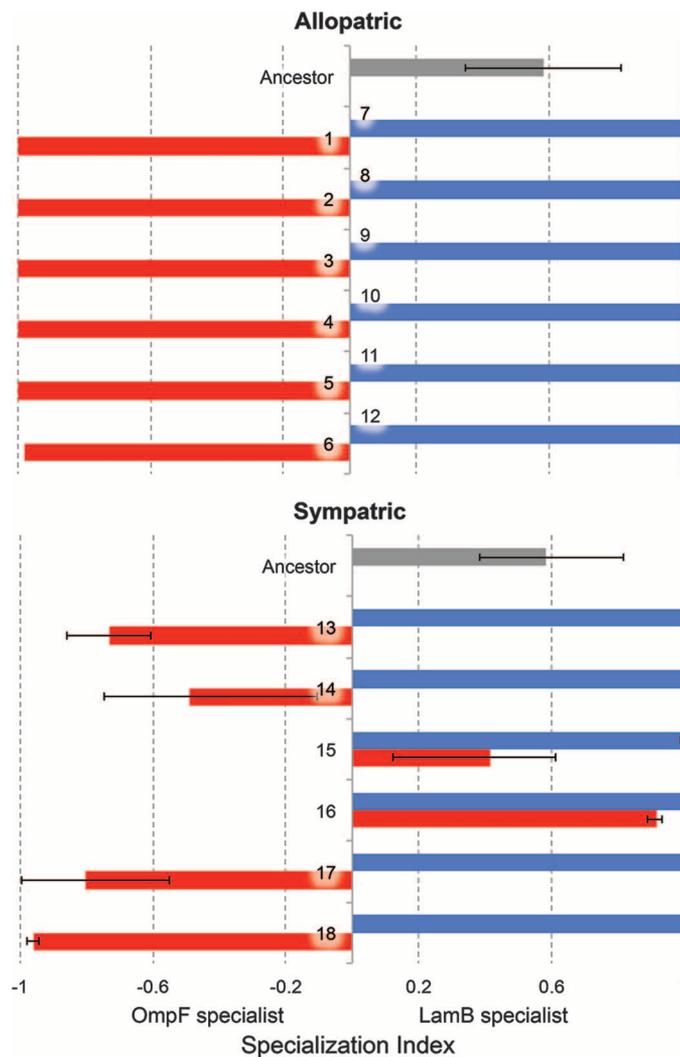
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Fig. 1. Specialization in allopatry and sympatry. Phage λ populations 1 to 6 evolved with OmpF-only host cells, populations 7 to 12 with LamB-only cells, and populations 13 to 18 with both types.

Specialization index = $(\text{plaques}_{\text{LamB}} - \text{plaques}_{\text{OmpF}}) / (\text{plaques}_{\text{LamB}} + \text{plaques}_{\text{OmpF}})$. Index values of 1 and -1 indicate complete LamB and OmpF specialization, respectively.

Values for populations 1 to 12 were measured using samples of the entire λ population. Using whole-population samples for populations 13 to 18 would hide the underlying diversity of specialists, so each bar shows the mean of six phages isolated on either OmpF-only (red bars) or LamB-only (blue bars) hosts. Error bars denote 95% confidence intervals.



propensities promote speciation when resources are patchily distributed (17–19).

We propagated six initially homogeneous and identical populations of λ strain EvoC for 35 cycles of dilution into host populations containing an equal mix of genotypes expressing either the LamB or OmpF receptor (20). The high initial ratio of viruses ($\sim 10^8$) to bacteria ($\sim 2 \times 10^6$) increases the opportunity for co-infection and recombination. After 8 hours, surviving bacteria were killed to stop the host population from evolving resistance, and a new cycle of viral reproduction was initiated by transferring 1% of the phage into a fresh population of naive bacteria. Twelve other phage populations were also cultured with a single host type (half expressing only LamB and half expressing only OmpF). We describe these single-host populations as allopatric because the separate flasks prevent viruses that use different hosts from recombining at any point in time. We call the mixed-host treatments sympatric because viruses that infect the different host types in one generation may infect the same host and recombine in the next generation. One caveat to this

terminology is that viral recombination can only occur within host cells, thereby introducing a subtle fine-scale spatial element to the sympatric treatment.

Strong receptor specialization evolved in all 12 single-host populations (Fig. 1). All six phage populations (7 to 12) that evolved with only the LamB-expressing hosts could no longer produce plaques on OmpF cell lawns (20). Five of six populations (1 to 5) propagated on only the OmpF-expressing cells evolved similar specialization; one population (6) continued to produce plaques on LamB hosts but at lower levels than on OmpF hosts. The ancestral phage EvoC produced plaques on both hosts, although more on the type expressing the LamB receptor (Fig. 1).

To determine whether phage performance on the two hosts also diverged when phages evolved on mixed hosts, we isolated six phages that produced plaques on LamB hosts and six on OmpF hosts from each sympatric-treatment population. We performed the same assay on these 72 isolates as on the single-host populations (Fig. 1). Four populations (13, 14, 17, and 18) evolved both OmpF

and LamB specialists, although most were not as specialized as those that evolved in allopatry. Two populations (15 and 16) contained a mixture of LamB specialists and generalists that, like the ancestral phage, could infect both LamB and OmpF hosts. Therefore, diversification occurred in sympatry as well as in allopatry.

Models of adaptive radiations indicate that ecological speciation requires a trade-off in the ability to use different resources (21). Moreover, the relation must be convex, such that a diminished ability to use one resource is outweighed by an improvement on the alternative. We measured the adsorption rates for the ancestor and two isolates from each sympatric population, one isolated on OmpF-only cells and the second on LamB-only cells (Fig. 2). In all six cases, the ancestral values fell below the line connecting the evolved isolates (sign test, one-tailed $P = 0.0156$). This pattern indicates that mutations that increase specialization provide greater gains in adsorption rate on the focal receptor than losses on the alternative one. With all else being equal, natural selection should favor specialization and drive diversification. Adsorption rate is an important component of phage fitness, but not every adsorption event necessarily leads to a productive infection. Nonetheless, the correlation in receptor specialization based on adsorption rates and plaque formation is highly significant (20) (fig. S1).

We sequenced the host-recognition gene, *J*, from one phage isolated on each host from the mixed-host populations, one from each OmpF-only population, and one from five of the six LamB-only populations (20). (Population 8 was not represented because the sample failed to preserve.) In total, we found 67 substitutions at 28 different positions. All were nonsynonymous and in the reactive region of the host-recognition J protein (22), indicating that they were likely adaptive (Fig. 3A). The LamB specialists had fewer mutations than the OmpF specialists (means of 1.41 and 4.54, respectively; $t = 9.93$, $df = 21$, two-tailed $P < 0.0001$). It is not surprising that the LamB specialists had fewer mutations, given that the most recent ancestor (EvoC) favored LamB and that the ancestor of EvoC (λ strain cI26) only used LamB. In general, the evolved LamB specialists did not reacquire that preference via reversion; only 1 of the 12 sequenced alleles reverted at a single site of the five that distinguish EvoC from cI26 (a2988c in population 17). Only two sites had mutations in both LamB and OmpF specialists, whereas 10 sites had parallel substitutions across independently evolved specialists of the same type.

A cluster analysis performed on the basis of the genetic distance among *J* alleles shows the different pattern of substitutions between the LamB and OmpF specialists (20) [analysis of similarities (ANOSIM) $R = 0.659$, $P = 0.0001$ (Fig. 3B)]. Phages isolated on the same host genotype but from different flasks had more similar *J* alleles than phages isolated from the same flask but specialized on different hosts. By contrast, there are no compelling differences between phages specialized on the same host type but

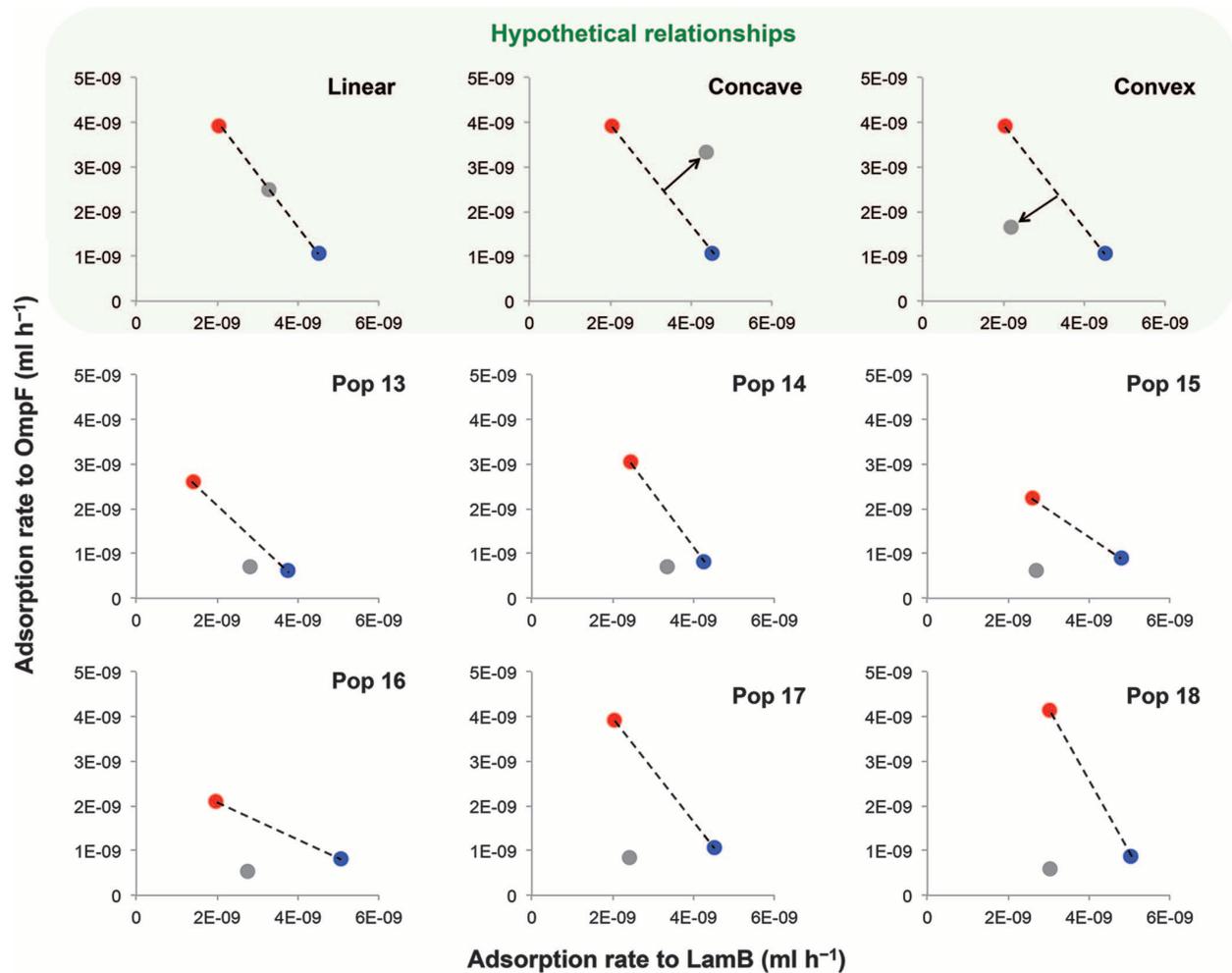


Fig. 2. Adsorption rates for two isolates from each sympatric population and the ancestor. The three top panels show hypothetical locations of the ancestor relative to evolved isolates. The six panels below show the data for each population that evolved on mixed hosts. Red symbols indicate phages isolated on OmpF-only hosts, blue symbols indicate phages isolated on LamB-only hosts, and gray symbols show the ancestor (EvoC). The ancestral rates were separately measured for each panel to ensure independence. See text for statistical analysis.

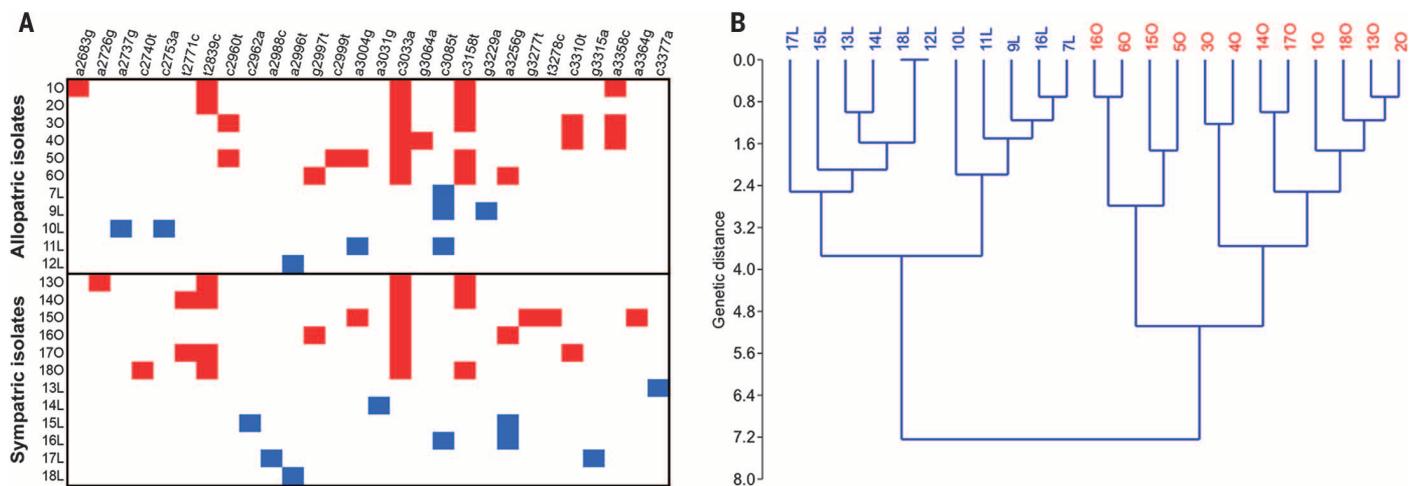
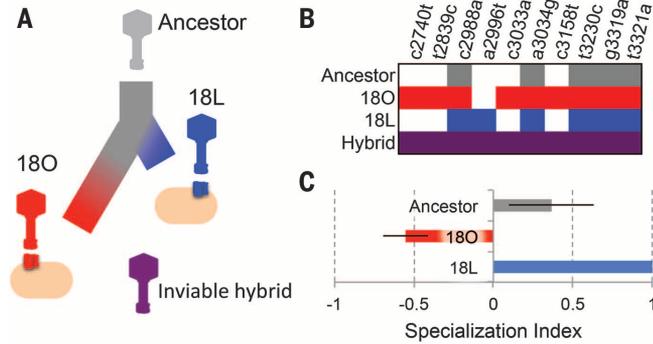


Fig. 3. Genetic signatures of ecological diversification. (A) Substitutions in the *J* gene from λ isolated from allopatric and sympatric treatments. Rows are λ isolates; columns are mutations. Red and blue fill shows mutations in phages isolated on OmpF-only and LamB-only hosts, respectively. (B) Cluster analysis using Ward's method separates alleles from independently evolved phages isolated on OmpF-only and LamB-only hosts, regardless of allopatric or sympatric treatment.

Fig. 4. Phage genotypes engineered to test interaction of mutations from *OmpF* and *LamB* specialists.

(A) Schematic of phage diversification in population 18 and the engineered hybrid of the two specialists. (B) Engineered *J* alleles. Colored fill shows mutations in engineered phages relative to the lysogenic phage background, *ci857*, used to engineer the four alleles shown. (C) Specialization index from plaque assays for the ancestor and two engineered specialists. The engineered hybrid with one *LamB*- and four *OmpF*-specific mutations did not produce plaques on either host. Error bars indicate 95% confidence intervals.



evolved in allopatry versus sympatry (20) (ANOSIM $R = 0.075$, $P = 0.1336$). These patterns indicate that selection in the two-host sympatry treatment drove the generalist phage populations to diverge into two subpopulations with distinctive genomes and preferences for the different receptors.

Another layer of reproductive isolation may arise along with host preference, if the mutations for *LamB* and *OmpF* specialization are incompatible. The Dobzhansky-Muller model predicts that speciation will occur if hybrids have lower fitness than their parents, reducing effective gene flow (23). To examine this possibility, we focused on population 18, where the *LamB* and *OmpF* specialists had one and four mutations, respectively, in the *J* gene (Fig. 3A). Using multiplexed automated genome engineering (MAGE) (20, 24) to edit a lysogenic strain of phage, *ci857*, we constructed alleles corresponding to the ancestral *EvoC* strain, the *LamB* specialist, the *OmpF* specialist, and a hybrid with all five mutations from the two specialists (Fig. 4, A and B). As expected, the constructed strains with the evolved alleles were specialists, and the one with the *EvoC* allele was a generalist (Fig. 4C). These observations indicate that the mutations in *J* are responsible for most, if not all, of the divergence in receptor preferences. Whole-genome sequencing of the specialists 18O and 18L revealed four additional mutations, but they occur in genes unlikely to affect interactions with the host receptor (20). Sympatric speciation is more feasible when a single gene has a large effect on ecological differentiation because it reduces the extent to which recombination could prevent divergence. Other studies of newly formed species have also found that variation in one or a few genomic regions accounts for most reproductive isolation (25).

Our first attempt to construct a hybrid between the *LamB* and *OmpF* specialists produced no viable phage, which suggests that the hybrid might not be viable. Our second attempt, however, produced a few infectious phages. Given this discrepancy, we hypothesized that the viable phages may have resulted from reversion or compensatory mutations that occurred during the

MAGE procedure. To test this, we sequenced the reactive region of *J* for five randomly chosen hybrids. All five had reverted the a2996t mutation to the ancestral state, removing the single *LamB*-specialization mutation and thereby recreating the *OmpF*-specialized allele. Thus, we found no viable true hybrids, providing evidence that the mutations that encode *OmpF* and *LamB* specialization are incompatible.

Our results show that λ likely evolved two mechanisms of reproductive isolation: (i) divergent host use leading to positive assortative recombination and (ii) genetic incompatibilities. These barriers satisfy the criteria for the biological species concept (5, 26). The distinctive ecological niches and genomic changes in the specialist phages also fulfill some other species definitions (27, 28). However, the specialists fall far short of a widely used criterion in phage taxonomy, namely <70% sequence similarity (28). We take this discrepancy to mean that we have observed the essential processes that can lead to speciation, but they have not operated long enough for the specialists to be classified as distinct species.

Four features of the experimental system contributed to the success of sympatric speciation and the speed with which it occurred. First, the convex trade-off between exploiting the two hosts set the stage for specialists to evolve. Second, the homogenizing effect of recombination was limited because a few mutations in a single gene accomplished this specialization. Third, the connection between host use and reproduction generated a barrier to gene flow as a by-product of natural selection. Fourth, the mutations that improved use of the alternative receptors were incompatible, preventing gene flow between the lineages. Determining how widespread these features are in nature remains an important empirical challenge. Understanding which features, if any, can be relaxed without disrupting speciation is both a theoretical and an empirical challenge.

REFERENCES AND NOTES

1. D. Schluter, *The Ecology of Adaptive Radiation* (Oxford Univ. Press, 2000).

2. R. Kassen, *Experimental Evolution and the Nature of Biodiversity* (Roberts, 2014).
3. P. B. Rainey, M. Travisano, *Nature* **394**, 69–72 (1998).
4. S. Gavrillets, *J. Hered.* **105** (suppl. 1), 743–755 (2014).
5. E. Mayr, *Animal Species and Evolution* (Belknap Press, 1963).
6. M. Barluenga, K. N. Stölting, W. Salzburger, M. Muschick, A. Meyer, *Nature* **439**, 719–723 (2006).
7. J. L. Feder, C. A. Chilcote, G. L. Bush, *Nature* **336**, 61–64 (1988).
8. C. H. Martin et al., *Evolution* **69**, 1406–1422 (2015).
9. J. R. Meyer et al., *Science* **335**, 428–432 (2012).
10. T. Baba et al., *Mol. Syst. Biol.* **2**, 2006.0008 (2006).
11. A. A. Lindberg, *Annu. Rev. Microbiol.* **27**, 205–241 (1973).
12. A. R. Poteete, *FEMS Microbiol. Lett.* **201**, 9–14 (2001).
13. A. T. Peterson, *Virology* **11**, 131 (2014).
14. G. A. Schachtel, P. Bucher, E. S. Mocarski, B. E. Blaisdell, S. Karlin, *J. Mol. Evol.* **33**, 483–494 (1991).
15. E. C. Bronson, J. N. Anderson, *J. Mol. Evol.* **38**, 506–532 (1994).
16. D. R. Forsdyke, *Microbes Infect.* **16**, 96–103 (2014).
17. S. Duffy, C. L. Burch, P. E. Turner, *Evolution* **61**, 2614–2622 (2007).
18. M. R. Servedio, G. S. Van Doorn, M. Kopp, A. M. Frame, P. Nosil, *Trends Ecol. Evol.* **26**, 389–397 (2011).
19. K. E. Filchak, J. B. Roethele, J. L. Feder, *Nature* **407**, 739–742 (2000).
20. Materials and methods and supplementary text are available as supplementary materials on Science Online.
21. M. Doebeli, *Adaptive Diversification* (Princeton Univ. Press, 2011).
22. S. Chatterjee, E. Rothenberg, *Viruses* **4**, 3162–3178 (2012).
23. D. R. Matute, I. A. Butler, D. A. Turissini, J. A. Coyne, *Science* **329**, 1518–1521 (2010).
24. H. H. Wang et al., *Nature* **460**, 894–898 (2009).
25. H. D. Bradshaw Jr., S. M. Wilbert, K. G. Otto, D. W. Schemske, *Nature* **376**, 762–765 (1995).
26. Q. Wheeler, R. Meier, *Species Concepts and Phylogenetic Theory: A Debate* (Columbia Univ. Press, 2000).
27. D. J. Howard, S. H. Berlocher, *Endless Forms: Species and Speciation* (Oxford Univ. Press, 1998).
28. M. Achtman, M. Wagner, *Nat. Rev. Microbiol.* **6**, 431–440 (2008).

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SUPPLEMENTARY MATERIALS

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Supplementary Text

Fig. S1

Tables S1 and S2

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Ecological speciation of bacteriophage lambda in allopatry and sympatry

Justin R. Meyer, Devin T. Dobias, Sarah J. Medina, Lisa Servilio, Animesh Gupta and Richard E. Lenski (November 24, 2016)
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Editor's Summary

A laboratory model of viral speciation

New species arise through the evolution of barriers to reproduction. This process is well understood where the emerging species are spatially isolated from one another. But the process of sympatric speciation—where the diverging species co-occur—is more enigmatic. Bacterial viruses make good models for addressing such questions because of their rapid generation times. Meyer *et al.* took bacteriophage λ and grew it on a mix of two strains of *Escherichia coli*. The virus duly split its host preferences into lineages with differing affinity for their respective *E. coli* hosts. In some experiments, the phages diverged to the extent that they became reproductively restricted to one strain of *E. coli*, even when the other was present.

Science, this issue p. 1301

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