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COMPETITION AND THE EVOLUTION OF NOVEL RESOURCE USE: AN EXPERIMENTAL TEST OF A VIRUS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the College of Arts and Sciences.

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ABSTRACT

Lisa Mullen Bono: Competition and the evolution of novel resource use: an experimental test in a virus
(Under the direction of Christina Burch and David Pfennig)

Competition for resources has long been hypothesized to be a key agent of diversification: individuals utilizing a novel resource have an advantage when competition for a preferred resource is strong. Over time, competition could drive the evolution of alternative resource-use phenotypes and potentially new species. Here, we use an experimental evolution approach to establish a direct link between competition and evolution and maintenance of diversity in the bacteriophage (bacteria-infecting virus) φ6. First, we demonstrated that reducing the availability of a standard host (a vital resource) drives the origin of novel host use by selecting for a phenotype with an expanded niche (generalist). However, generalists evolved without a detectable trade-off on the original, standard host and competitively excluded the standard host specialists in all but one population. Second, we tested if competition could drive the maintenance of diversity by enabling coexistence of generalists and specialists. By increasing the ratio of the standard to novel resources, we simultaneously decreased competition for the standard resource and reduced ecological opportunity. Sustained coexistence was more likely because specialists had more time to evolve a have higher fitness on the standard host, generating a trade-off in host performance. Third, we tested if the presence of a competitor could act as a wedge, driving generalists and a competitor phenotype (the specialist) to diverge in resource use in sympatry. As a control, we evolved the generalist alone on the novel resource in allopatry.

However, sympatric generalists adapted to the standard host while allopatric generalists declined on the standard host. Sympatric generalists evolved to take advantage of both hosts and minimized antagonistic pleiotropy, while allopatric generalists evolved in the absence of such selection and thereby decreased in standard host adsorption. Overall, direct costs to expanded host range remained difficult or elusive to detect, despite previous studies documenting antagonistic pleiotropy in φ6. Rather, generalists consistently evolved with both host experienced selection to minimize antagonistic pleiotropy, which has serious implications for theory that uses these costs as the basis for divergence. Taken together, these results show strong support for the hypothesis that competition is a key agent of diversification.

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CHAPTER 1: INTRODUCTION

> "The truth of the principle, that the greatest amount of life can be supported by great diversification of structure, is seen under many natural circumstances. In an extremely small area…and where the contest between individual and individual must be severe, we always find great diversity in its inhabitants."
> --Charles Darwin (1859)

> "The observations I refer to were made with a view to discovering whether it was possible by change of environment, in minute life-forms, whose life-cycle was relatively soon completed, to superinduce changes of an adaptive character, if the observations extended over a sufficiently long period."
> --William Dallinger (1887)

Unraveling the mystery of the tremendous diversity of living things requires an understanding of both the origin of novelty as well as how that novelty is maintained after it evolves. Competition for resources has long been hypothesized to be a key agent of diversification: individuals utilizing a novel resource have an advantage when competition for a preferred resource is strong. Darwin (1859) was the first to attribute competition as being a key agent of diversification, selecting for individuals least like their competitors and then acting as a wedge to drive phenotypes apart in a distinctive "tree-like" topology. Competition for resources may be the most common of all selective forces (Vermeij 1993; Pfennig and Pfennig 2012), because competition, unlike other ecological interactions, is uniquely mutually costly to both parties and, therefore, a particularly potent agent of selection. When faced with competition for resources, an individual can either use the limiting resource faster than competitors, use an alternative resource, or die.

Although competitive exclusion was demonstrated by Gause (1934) early on, it is clear that competing species and alternative phenotypes coexist in nature. Coexistence can arise via niche partitioning by subdividing resources in a way that reduces competition. This idea was suggested by Darwin (1859) when he said, "…more living beings can be supported on the same area the more they diverge in structure, habits, and constitution." Niche partitioning can occur by temporally, spatially or phenotypically segregating resource acquisition. In this last case, phenotypic partitioning may lead to the evolution of different ecomorphs that specialize on different resources, e.g. *Anolis* lizards form ecomorphs that utilize different microhabitats to minimize competition on the Greater Antilles (Losos 2009). An alternative, non-mutually exclusive but non-adaptive explanation for resource partitioning is species sorting, which occurs when species that already display differences in resource acquisition invade or when species that are too similar to coexist go extinct (Pfennig and Pfennig 2012).

The conditions under which coexistence is possible have been shown using a variety of approaches. Differences in resource use promote coexistence when intraspecific competition is stronger than interspecific competition, because intraspecific competition can regulate a species population size, preventing it from becoming so numerous that it excludes the other species. This concept was elegantly demonstrated by Lotka (Lotka 1932) and Volterra (Volterra 1926) mathematically using equilibrium models. Alternative explanations use non-equilibrium models to show that coexistence occurs when the superior competitor is suppressed and therefore unable to reach its carrying capacity due to factors such as environmental variability, disturbance (Paine 1979) and exploitation (Paine 1966; Lubchenco 1978), thereby preventing extinction of the inferior competitor(s) (Hutchinson 1961). The niches of two species must be sufficiently different in order for them to coexist (Macarthur and Levins 1964) with the frequency of

competitive exclusion increasing with increasing similarity between traits (Levine and HilleRisLambers 2009).

Theory predicts that populations facing intense intraspecific competition tend to undergo niche width expansion, evolving to utilize a wider range of resources (Valen 1965; MacArthur et al. 1967; Roughgarden 2009). By evolving to consume an underutilized resource—even a resource that is novel, poor or toxic—individuals can escape the intense competition for the shared or preferred resource. Niche-width expansion comprises scenarios where phenotypes within a population specialize on different resources or generalize to consume a range of resources (Roughgarden 2009). Few direct studies provide direct empirical evidence of this process. In one notable exception *Drosophila melanogaster* was provided with food laced with differing levels of cadmium, which is toxic to flies. Despite being initially tolerant of cadmium, flies still oviposited on food laced with high levels of cadmium due to the low egg density, despite reduced performance. Mutations allowing cadmium-tolerance soon arose and increased in frequency. Furthermore, populations experiencing high levels of intraspecific competition evolved cadmium-tolerance faster than those experiencing lower levels of competition (Bolnick 2001).

Adaptive responses to intense intraspecific competition can lead to continuous variation in resource use or a discrete polymorphism. In the latter case, alternative phenotypes that differ in resource use and can vary dramatically in physiology, morphology, behavior and life history, e.g. planktivory versus benthivory is a variety of fish species (Smith and Skulason 1996), omnivory versus carnivory in spadefoot toad tadpoles (Pfennig 1992), and preference in host plant type in phytophagous insects (Via et al. 2000). These alternative phenotypes can coexist within the same population stably when intense intraspecific competition for resources leads to

frequency-dependent disruptive selection. Competition can act as a wedge, driving phenotypes farther apart in niche space with disruptive selection acting on resource use traits (Calsbeek and Smith 2003; Bolnick 2004; Pfennig and Rice 2007; Pfennig and Martin 2009). With negative frequency-dependent selection, fitness is dynamic in relation to frequency with phenotypes having an advantage when rare but not common, e.g. handedness in a scale-eating cichlid (*Perissodus microlepis*, Hori, 1993), handedness in crossbills (*Loxia* spp., Benkman, 1996), and cannibalism in tiger salamander larvae (*Ambystoma tigrinum*, Maret & Collins, 1997). Notably, the alternative phenotypes may vary in terms of niche width with generalists and specialists coexisting, e.g. snail specialist molariform versus generalist papilliform in the cichlid *Herichthys minckleyi* (Robinson and Wilson 1998) and generalist omnivore versus specialist carnivore in *Spea* (Pfennig 2000; Ledón-Rettig and Pfennig 2011).

In this second scenario one morph specializes on the standard resource, which is higher quality, and generalists that can additionally utilize a novel and relatively poor resource, trade-offs allow for coexistence. Although the specialist monopolize the more profitable resource, it experiences intense competition for that resource. On the other hand, generalists experience competitive release when using the novel resource, but it may be less profitable (Pfennig and Pfennig 2012). Trade-offs can results from a direct cost on the generalist's ability to utilize the standard resource via antagonistic pleiotropy, i.e. a jack-of-all-trades is a master of none, or through a variety of indirect costs. For instance, by alternating resources, a generalist does not experience selection on the shared resource as often as a specialist and is, therefore, expected to adapt slower to the shared resource. A generalist could pay a fitness cost if it utilizes a relatively poor novel resource when a superior resource is available. Again, negative frequency-dependent selection can then lead to the coexistence of generalists and specialists.

A resource polymorphism may represent a critical, early step in speciation (West-Eberhard 2005; Mallet 2008; Hendry 2009). One piece of indirect evidence for this is that clades in which resource-use polymorphisms have evolved have higher species diversity than their sister clades that did not (Pfennig and McGee 2010). Specifically, as alternative resource-use phenotypes come to occupy separate niches, selection should favor traits that improve each phenotype's ability to survive and reproduce in its particular niche. As alternative morphs in a resource polymorphism become more distinct—ecologically, phenotypically and genetically—they may eventually become reproductively isolated from each other. Importantly, the evolution of reproductive isolation via divergent natural selection has been hypothesized to occur both with and without gene flow between the alternative phenotypes (West-Eberhard 2005; Mallet 2008; Hendry 2009; Pfennig and Pfennig 2012).

Most of the evidence that I have described so far has been indirect with relatively few studies directly linking competition and niche width expansion (see Bolnick (2001) for a notable exception). For my doctoral dissertation, I have sought to help fill this gap using a microbial experimental evolution approach, because it is a powerful method for studying fundamental questions in evolution and ecology by allowing for simple yet elegant experiments with a high degree of control and replication not possible in the field (Jessup et al. 2004; Kawecki et al. 2012). Additionally, microbes can be archived and revived at a later date, creating a frozen fossil record, so that an ancestor can be directly compared to its descendants using phenotypic assays and sequencing (Barrick and Lenski 2013). Within just a couple decades of the publication of the *Origin of Species* (Darwin 1859), William Dallinger began using microbes to study how ecological factors can influence evolution (Dallinger 1887). Microbial evolution experiments have proven a profitable model for studying diversification experimentally in real time, yielding

insight into the evolution of novel resource-use polymorphisms (Turner et al. 1996; Blount et al. 2008) and character displacement (Tyerman et al. 2008) in *Escherichia coli*, adaptive radiation in heterogeneous environments in *Pseudomonas fluorescens* (Rainey and Travisano 1998), and costs of niche width expansion in vesicular stomatitis virus (VSV, Turner and Elena 2000) and the bacteriophage φ6 (Duffy et al. 2006; Ferris et al. 2007).

Studying the evolution of novel resource use in microbes is particularly pertinent when considering pathogens. When a pathogen evolves to utilize a novel resource, it is evolving to infect or "jump into" a new species. RNA viruses in particular are highly likely to emerge into humans from zoonotic origins (Cleaveland et al. 2001; Woolhouse and Gaunt 2007), because they can take advantage of all known genetic mechanisms, including high mutation rate, high yields and short generation time, to evolve and adapt to new host species rapidly (Domingo and Holland 1997). Examples of zoonotic RNA viruses that have recently emerged into humans with serious implications to human health include Human Immunodeficiency Virus (HIV), Influenza A H1N1 in 1918 and 2009 (Cohen 2010; Xu et al. 2010), Ebola, Middle East Respiratory Syndrome (MERS), among many others. As viruses can evolve rapidly in response to host ecology, integrating evolutionary ecology can enhance predictions made over using traditional epidemiological approach alone (Galvani 2003).

In my dissertation, I used the bacteriophage φ6 to test experimentally the role of competition on the evolution of novel resource-use. φ6 is an RNA bacteriophage (bacteria-infecting virus) that infects *Pseudomonas syringae*, which itself is a plant pathogen. With its high mutation rate and short generation time, it has been a fruitful model for studying the nature of mutations and epistasis (Burch and Chao 1999, 2000, 2004; Duffy et al. 2007). φ6 has a variety of characteristics that make it well-suited to test the role of competition on novel resource-use.

First, previous studies have shown that it can rapidly evolve to expand its host range, infecting a variety of pseudomonad strains and species (Cuppels et al. 1980) and therefore has been productive for studying the nature of host range mutations (Duffy et al. 2006; Ferris et al. 2007). Second, as a segmented virus, gene flow occurs via reassortment and has been a powerful system to study the evolution of sex (Chao 1990; Chao et al. 1992, 1997; Turner and Chao 1998). Additionally, φ6 can evolve to use a novel host exclusively, restricting its host range and becoming reproductively isolated from its ancestors (Duffy et al. 2007). Third, the strength of competition can be altered by adjusting the phage-to-host ratio (multiplicity of infection, MOI) or ratio of standard and novel hosts. In this manner, I used φ6 to study how competition can drive novel host use, maintain alternative resource-use phenotypes, as well as how the presence of a competitor affects adaptation to a novel resource.

REFERENCES

Barrick, J. E., and R. E. Lenski. 2013. Genome dynamics during experimental evolution. Nat. Rev. Genet. 14:827–39.

Benkman, C. W. 1996. Are the ratios of bill crossing morphs in crossbills the result of frequency-dependent selection? Evol. Ecol. 10:119–126.

Blount, Z. D., C. Z. Borland, and R. E. Lenski. 2008. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. 105:7899–906.

Bolnick, D. I. 2004. Can intraspecific competition drive disruptive selection ? An experimental test in natural populations of sticklebacks. Evolution. 58:608–618.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410:463–6.

Burch, C. L., and L. Chao. 2004. Epistasis and its relationship to canalization in the RNA virus phi 6. Genetics 167:559–67.

Burch, C. L., and L. Chao. 1999. Evolution by small steps and rugged landscapes in the RNA virus φ6. Genetics 151:921–7.

Burch, C. L., and L. Chao. 2000. Evolvability of an RNA virus is determined by its mutational neighbourhood. Nature 406:625–8.

Calsbeek, R., and T. B. Smith. 2003. Ocean currents mediate evolution in island lizards. Nature. 426:4–7.

Chao, L. 1990. Fitness of RNA virus decreased by Muller's ratchet. Nature 348:454–455.

Chao, L., T. Tran, and C. Matthews. 1992. Muller's ratchet and the advantage of sex in the RNA virus φ6. Evolution. 46:289–299.

Chao, L., T. T. Tran, and T. T. Tran. 1997. The advantage of sex in the RNA virus. Evolution. 147:953–959.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 356:991–999.

Cohen, J. 2010. Swine flu pandemic. What's old is new: 1918 virus matches 2009 H1N1 strain. Science 327:1563–1564.

Cuppels, D. a, J. L. Van Etten, D. E. Burbank, L. C. Lane, and a K. Vidaver. 1980. In vitro translation of the three bacteriophage φ6 RNAs. J. Virol. 35:249–51.

Dallinger, W. H. 1887. The President's Address. J. R. Microsc. Soc. 7:185–199.

Darwin, C. 1859. *On the origin of species by Charles Darwin: A facsimile of the first edition with an introduction by Ernst Mayr*. Harvard University Press.

Domingo, E., and J. J. Holland. 1997. RNA virus mutations and fitness for survival. Annu. Rev. Microbiol. 51:151–78.

Duffy, S., C. L. Burch, and P. E. Turner. 2007. Evolution of host specificity drives reproductive isolation among RNA viruses. Evolution 61:2614–22.

Duffy, S., P. E. Turner, and C. L. Burch. 2006. Pleiotropic costs of niche expansion in the RNA bacteriophage φ6. Genetics 172:751–7.

Ferris, M. T., P. Joyce, and C. L. Burch. 2007. High frequency of mutations that expand the host range of an RNA virus. Genetics 176:1013–22.

Galvani, A. P. 2003. Epidemiology meets evolutionary ecology. Trends Ecol. Evol. 18:132–139.

Gause, G. F. 1934. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. Science 79:16–17.

Hendry, A. P. 2009. Ecological speciation! Or the lack thereof? Can. J. Fish. Aquat. Sci. 66:1383–1398.

Hori, M. 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. Science 260:216–219.

Hutchinson, G. E. 1961. The paradox of the plankton. Am. Nat. 95:137–145.

Jessup, C. M., R. Kassen, S. E. Forde, B. Kerr, A. Buckling, P. B. Rainey, and B. J. M. Bohannan. 2004. Big questions, small worlds: microbial model systems in ecology. Trends Ecol. Evol. 19:189–97.

Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012. Experimental evolution. Trends Ecol. Evol. 27:547–60.

Ledón-Rettig, C. C., and D. W. Pfennig. 2011. Emerging model systems in eco-evo-devo: The environmentally responsive spadefoot toad. Evol. Dev. 13:391–400.

Levine, J. M., and J. HilleRisLambers. 2009. The importance of niches for the maintenance of species diversity. Nature 461:254–7.

Losos, J. 2009. Lizards in an Evolutionary Tree. University of California Press, Berkeley, CA.

Lotka, A. J. 1932. The growth of mixed populations: two species competing for a common food supply. J. Washingt. Acad. Sci. 22:461–469.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. Am. Nat. 112:23–39.

Macarthur, B. Y. R., and R. Levins. 1964. Competition, habitat selection, and character displacement in a patchy environment. Proc. Natl. Acad. Sci. U. S. A. 51:1207–1210.

MacArthur, R. H., E. O. Wilson, and W. MacArthur. 1967. The theory of island biogeography. Princeton University Press.

Mallet, J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363:2971–2986.

Maret, T. T., and J. P. Collins. 1997. Ecological Origin of Morphological Diversity: A Study of Alternative Trophic Phenotypes in Larval Salamanders. Evolution. 51:898–905.

Paine, R. T. 1979. Disaster, Catastrophe, and Local Persistence of the Sea Palm *Postelsia palmaeformis*. Science 205:685–7.

Paine, R. T. 1966. Food Web Complexity and Species Diversity. Am. Nat. 100:65–75.

Pfennig, D. W. 2000. Effect of predator-prey phylogenetic similarity on the fitness consequences of predation: a trade-off between nutrition and disease? Am. Nat 155:345–355

Pfennig, D. W. 1992. Polyphenism in spadefoot toad tadpoles as a logically adjusted evolutionarily stable strategy. Evolution 46:1408–1420.

Pfennig, D. W., and R. A. Martin. 2009. A maternal effect mediates rapid population divergence and character displacement in spadefoot toads. Evolution 63:898–909.

Pfennig, D. W., and M. McGee. 2010. Resource polyphenism increases species richness: a test of the hypothesis. Philos. Trans. R. Soc. London, Ser. B 365:577–591.

Pfennig, D. W., and K. S. Pfennig. 2012. Evolution's Wedge: Competition and the Origins of Diversity. University of California Press, Berkeley, CA.

Pfennig, D. W., and A. M. Rice. 2007. An experimental test of character displacement's role in promoting postmating isolation between conspecific populations in contrasting competitive environments. Evolution. 61:2433–43.

Rainey, P. B., and M. Travisano. 1998. Adaptive radiation in a heterogeneous environment. Nature 32:69–72.

Robinson, B. W., and D. S. Wilson. 1998. Optimal foraging, specialization, and a solution to Liem's paradox. Am. Nat. 151:223–35.

Roughgarden, J. 2009. Evolution of niche width. Am. Nat. 106:683–718.

Smith, T. B., and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu. Rev. Ecol. Syst. 27:111–133.

Turner, P. E., and L. Chao. 1998. Sex and the evolution of intrahost competition in RNA virus φ6. Genetics 150:523–32.

Turner, P. E., and S. F. Elena. 2000. Cost of host radiation in an RNA virus. Genetics 156:1465–70.

Turner, P. E., V. Souza, and R. E. Lenski. 1996. Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology 77:2119–2129.

Tyerman, J. G., M. Bertrand, C. C. Spencer, and M. Doebeli. 2008. Experimental demonstration of ecological character displacement. BMC Evol. Biol. 8:34.

Valen, L. Van. 1965. Morphological variation and width of ecological niche. Am. Nat 99:377–399

Vermeij, G. J. 1993. Evolution and Escalation: An Ecological History of Life. Princeton University Press.

Via, S., A. C. Bouck, and S. Skillman. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. Evolution. 54:1626–1637.

Volterra, V. 1926. Fluctuations in the abundance of a species considered mathematically. Nature 118:558–560.

West-Eberhard, M. J. 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. 102:6543–6549.

Woolhouse, M., and E. Gaunt. 2007. Ecological origins of novel human pathogens. Crit. Rev. Microbiol. 33:231–242.

Xu, R., D. C. Ekiert, J. C. Krause, R. Hai, and I. A. Wilson. 2010. Structural basis of preexisting immunity to 2009 H1N1 pandemic influenza virus. Science. 328:358–360.

CHAPTER 2: COMPETITION AND THE ORIGINS OF NOVELTY: EXPERIMENTAL EVOLUTION OF NICHE-WIDTH EXPANSION IN A VIRUS

Summary

Competition for resources has long been viewed as a key agent of divergent selection. Theory holds that populations facing severe intraspecific competition will tend to utilize a wider range of resources, possibly even utilizing entirely novel resources that are less in demand. Yet, there have been few experimental tests of these ideas. Using the bacterial virus (bacteriophage) ϕ6 as a model system, we examined whether competition for host resources promotes the evolution of novel resource use. In the lab, ϕ6 exhibits a narrow host range, but readily produces mutants capable of infecting novel bacterial hosts. Here, we show that when ϕ6 were subjected to intense intraspecific competition for their standard laboratory host, they rapidly evolved new generalist morphs that infect novel hosts. Our results therefore suggest that competition for host resources may drive the evolution of host range expansion in viruses. More generally, our findings demonstrate that intraspecific resource competition can indeed promote the evolution of novel resource-use phenotypes.

Introduction

The appearance of novel resource-use phenotypes characterizes most adaptive radiations, and intraspecific competition is thought to play a key role in this process (Simpson 1953; Schluter 2000). Specifically, theory holds that in a population experiencing intense competition,

frequency-dependent selection will favor individuals that can exploit an underutilized resource—even if this resource is novel and of lower quality—because these individuals will experience decreased competition (Rosenzweig 1978; Wilson and Turelli 1986). Such selection has long been viewed as crucial in promoting niche-width expansion (Bolnick 2001), resource polymorphism (Smith and Skúlason 1996) and even speciation and adaptive radiation (Schluter 2000; Pfennig and McGee 2010). Yet, much of the empirical support for this theory is indirect (Schluter 2000; Pfennig and McGee 2010). Indeed, relatively few experimental studies have established a causal link between resource competition and niche-width expansion (Bolnick 2001).

Here, we present such an experimental test. Using a rapidly evolving bacteriophage, ϕ6 (a virus that infects bacteria), we determined whether novel resource (host) use was more likely to evolve, and evolve more rapidly, when populations experienced greater competition for hosts. Additionally, we tested three predictions of competition theory: (1) that rare resource-use phenotypes would be favored over common ones, yielding frequency-dependent selection (Doebeli 2011); (2) that generalists utilizing both the ancestral host and a novel host would have lower fitness than specialists on the ancestral host, owing to fitness trade-offs and lower quality of novel resources (Bolnick 2001); and (3) that novel resource use would evolve faster in larger populations, owing to a greater supply of adaptive mutations (Barrett and Schluter 2008). Although we found support for only some of these predictions, overall, our results suggest that intraspecific resource competition does indeed promote the evolution of novel resource-use phenotypes.

Experimental Design.

Phage populations were evolved using serial transfer into fresh bacterial cultures containing a 1:1 mixture of the standard laboratory host and a novel (non-permissive) host at a total density of 10^8 cells/mL (diagrammed in Figure 1). Individual lineages were subjected to either strong (Figure 1A-1C) or weak (Figure 1D-1F) intraspecific competition by initiating each transfer at a ratio of phage to hosts (multiplicity of infection or MOI), of 10^{-3} or 10^{-1}, respectively. This increase in MOI reduces the intrinsic growth rate of ϕ6 ($ln(P_6/P_0)$; see methods) from 12.7 to 8.4 per transfer (Appendix A). Individual lineages were propagated using transfer population sizes of $N = 10^5$, 10^6 or 10^7 phage (determined by plating at the end of each transfer). Total culture volume was adjusted between 10 µL (Figure 1A) to 100 mL (Figure 1F) to achieve a constant host density across treatments despite differences in MOI and phage population size. Cultures were incubated shaking at 25°C for 6 hours and filtered to remove host cells, and a sample of 10^5, 10^6 or 10^7 of the resulting phage was used to initiate the next transfer cycle. This protocol was repeated for 20 transfers.

We monitored evolution in independent microcosms containing one of 3 novel hosts – *P. syringae* pathovar *glycinea*, *P. syringae* pathovar *atrofaciens*, or *P. pseudoalcaligenes* pathovar ERA. These hosts were chosen because ϕ6 requires only a single point mutation to infect them (Duffy et al. 2006, Ferris 2007). In laboratory culture, their growth rates differ (doublings/hour = 0.19 on *Ps glycinea*, 0.26 on *Ps atrofaciens*, and 0.45 on *Pp ERA*, compared to 0.35 on the standard host *Ps phaseolicola*), and generalist mutant phage exhibit different growth rates on three hosts. The median (± s.d.) intrinsic growth rate of three independent generalist mutants ranged from 7.80 ± 0.59 on *Ps glycinea* to 5.78 ± 4.46 on *Pp ERA* to 3.93 ± 1.27 on *Ps atrofaciens*. Thus, the novel hosts differ in quality with *Ps glycinea* > *Pp ERA* > *Ps atrofaciens*.

Materials and Methods

(a) ***Strains and Culture Conditions*****.** The RNA bacteriophage ϕ6 used in this study is a laboratory strain descended from the original isolate (Vidaver et al. 1973). The bacterium *Pseudomonas syringae* pathovar *phaseolicola* strain HB10Y (Mindich et al. 1976) served as the standard host. Novel host strains included *P. syringae* pathovar *atrofaciens* 2231, *P. syringae* pathovar *glycinea* 171 (obtained from Greg Martin, Cornell University) and *P. pseudoalcaligenes* pathovar ERA (Mindich et al. 1976). Culture conditions are described in the electronic supplementary material.

(b) ***Competition and Growth Rate Assays.*** We measured the growth rates of evolved generalist and specialist phage both when grown together in direct competition on mixtures of the standard and novel hosts and when grown in isolation on the standard host only. Relative fitness was calculated from competition assays as $ln(R_6/R_0)$, where R_t is the ratio of generalist to specialist phage at t hours. Intrinsic growth rate was calculated from cultures containing only specialists or only generalists as $ln(P_6/P_0)$, where P_t is the concentration of phage at t hours. The assay conditions mimicked the evolution experiments described in Figure 1 (see Appendix A).

(c) ***Attachment Rate Assays.*** We measured the rate at which phage bind to the standard and novel hosts (see Appendix A).

(d) *Statistical Analysis*. All statistical analyses were performed in R v. 2.13.0. In addition to standard linear models and t-tests, we used a survival analysis (function *survreg*) to compare the time at which generalists arose across treatments (see Appendix A).

(e) Data archive. All data are archived at datadryad.org.

Results

As predicted, when ϕ6 were subjected to intense intraspecific competition for their standard laboratory host, they rapidly evolved a new generalist morph that infected novel hosts (Figure 2). The rate at which generalists evolved depended on the novel host ($\chi^2 = 21.60$, df = 2, $p < 0.0002$; full analysis in Table 1) with generalists arising fastest on *Ps glycinea* and slowest on *Ps atrofaciens*. In every case, generalists arose more often and earlier when competition was strong than when it was weak ($\chi^2 = 5.85$, df = 1, $p = 0.0156$). However, we did not find an effect of population size in this initial set of lineages.

We expected population size to affect the timing of generalist evolution through its impact on mutation supply. Therefore, we provided a more powerful test of the effect of population size by evolving four additional replicate lineages under strong competition for each population size in microcosms containing *Ps atrofaciens* or *Pp ERA* (the hosts that showed the strongest difference between the strong and weak competition treatments). We again detected no effect of population size on the time at which generalists first appeared (Figure 3; Table 1; $\chi^2 = 1.57$, df = 2, $p = 0.4551$).

We examined the stability of the coexistence between specialists and generalists on *Ps atrofaciens* or *Pp ERA* using competition experiments. We measured the fitness of evolved

generalists relative to evolved specialists taken from each lineage in which the generalist had not competitively excluded the ancestral specialist by the 20th transfer. We found that fitness was frequency-dependent, with generalists exhibiting a higher mean relative fitness when rare than when common (Welch two sample t-test $t_{121.208}$=7.457 p = 7.31x10^{-12}; Figure 4). However, the high fitness of generalists when common (ln(W) ≥ 0 using Fisher's Least Significant Difference) indicates that generalists would eventually exclude the specialist in all but one lineage.

Generalists had higher fitness than specialists because expanded host range was only rarely associated with reduced growth on the standard host. When we measured intrinsic growth rate on the standard host, we found that generalists tended to have lower growth rates than specialists overall (p = 0.0037, $F_{1,250}$ = 8.592; Table 3), but this overall difference was due to a large fitness cost paid by generalists in only a few lineages (2/13 on *Ps atrofaciens* and 4/14 on *Pp ERA*, Appendix A).

To further explore the reasons that generalists tended to competitively exclude specialists, we also tested whether the evolved generalists attached to (and infected) the novel host only rarely. We measured attachment rates (k) of generalist phage sampled from three independent populations evolved on each of the three novel host treatments. Based on these measures we estimate that seven of these nine generalists were significantly (p<0.05) less likely to infect the novel host than the standard host (mean probability of infecting the novel host Pr = $k_{novel}/(k_{standard}+k_{novel})$ = 0.26 for *Pp ERA*, 0.04 for *Ps atrofaciens*, and 0.34 for *Ps glycinea*, see table 4). Only one *Pp ERA* lineage was significantly more likely to infect the novel host (Pr = 0.72; p = 0.0009).

Discussion

Intraspecific competition for resources has long been regarded as a key agent of divergent selection (Schluter 2000). In some cases, competition may even promote the evolution of novel resource-use phenotypes, although few experiments have demonstrated this (Bolnick 2001). We sought to fill this gap by using a rapidly evolving bacteriophage, ϕ6. We predicted that novel host use would be more likely to evolve, and evolve more rapidly, when populations experienced greater competition for hosts.

As predicted, when ϕ6 were subjected to strong intraspecific competition for their standard host, they rapidly evolved a new generalist morph capable of infecting a novel host Figure 2. Generalists arose earliest on the highest quality novel host *Ps glycinea* and latest on the lowest quality novel host *Ps atrofaciens*. Under weak competition, however, generalists evolved only on *Ps glycinea.* Even then, generalists evolved much later than in the strong competition treatment for this host. The rate at which generalists arose did not depend on population size (Figure 3), indicating that genetic variation (i.e. mutation supply) was not limited even in our small populations of size $N=10^5$. Experimental design constraints prevented us from examining smaller populations.

We also anticipated that generalists would possess higher mean relative fitness when rare than when common. Such negative frequency-dependent fitness is a hallmark of competitive mediated selection (Friesen et al. 2004). Although fitness was indeed frequency-dependent (Figure 3), we saw little evidence that frequency-dependent selection was strong enough to enable generalists and specialists to coexist. Instead, in most populations, generalists outperformed specialists both when rare and common. Indeed, generalists generally grew as well on the standard host as did specialists. Moreover, generalists attached slowly to the novel host

and, therefore, rarely infected it when standard hosts were present in the microcosm. Future research will identify both the phenotypic and genetic bases of adaptations in these lineages to determine mechanistically why coexistence of generalists and specialists was rare.

The tendency of evolved generalists to competitively displace specialists in our experiments may be a consequence of the serial transfer regime used to propagate the phage populations. Serial transfers impose temporal variation of resource availability, which causes the intensity of competition to vary, thereby restricting the conditions for maintaining a stable polymorphism (Rainey et al. 2000). Here temporal variability was manifest as a change in the probability that a generalist phage infects a novel host over the timecourse of each serial transfer. In particular, early when the standard hosts were common, the generalist phage rarely infected the poorer quality novel hosts. As a result, generalists did not pay a cost for their ability to infect this host. Rather, they gained an advantage by infecting the poorer quality novel host only after the better quality standard host had been consumed. These data provide strong evidence that a phenotype that utilizes an untapped resource can persist—even when it is poorly adapted to that novel resource—if competition for the preferred resource is strong.

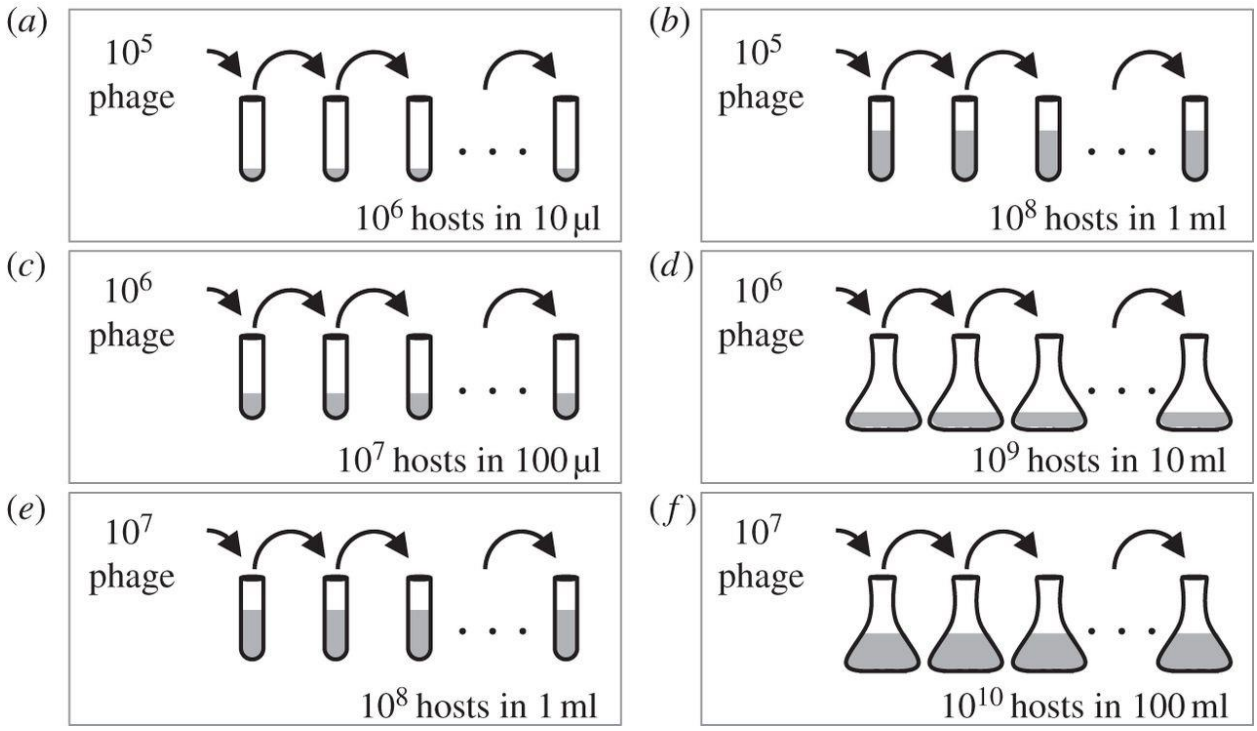

Figure 1. Experimental design.

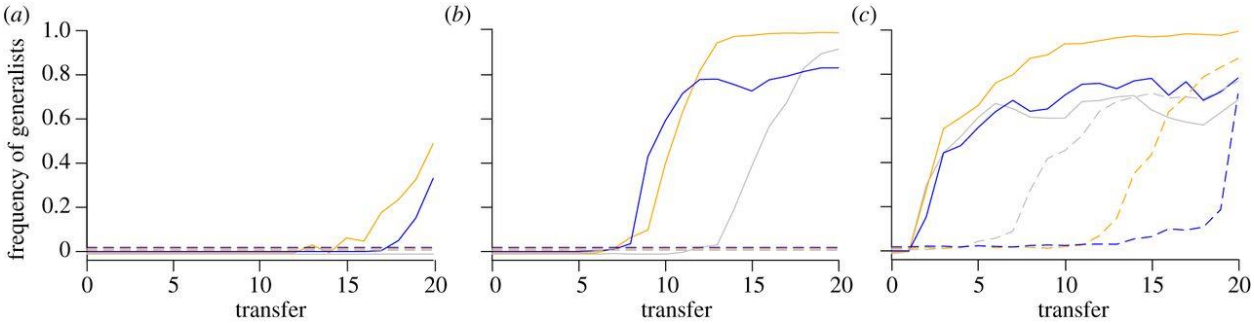

Figure 2. Effects of competition and novel host treatments on host range expansion.

Lines show the frequency of generalists over time in lineages evolved under weak (dashed lines) or strong (solid lines) competition, transfer population sizes of $N = 10^5$ (grey), 10^6 (orange) or 10^7 (blue), and novel host *Ps atrofaciens* (A), *Pp ERA* (B) or *Ps glycinea* (C). Lines are offset vertically to be distinguishable.

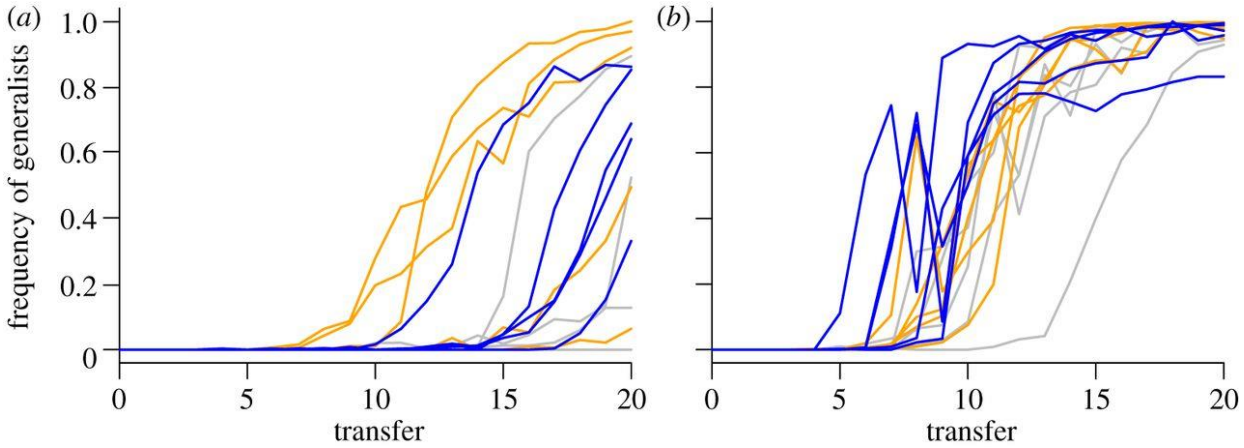

Figure 3. Effects of population size.

Effect of population size on host range expansion in microcosms containing novel hosts *Ps atrofaciens* (A) and *Pp ERA* (B). Lines show the frequency of generalists over time in lineages evolved at transfer population sizes of $N = 10^5$ (gray), 10^6 (orange) or 10^7 (blue).

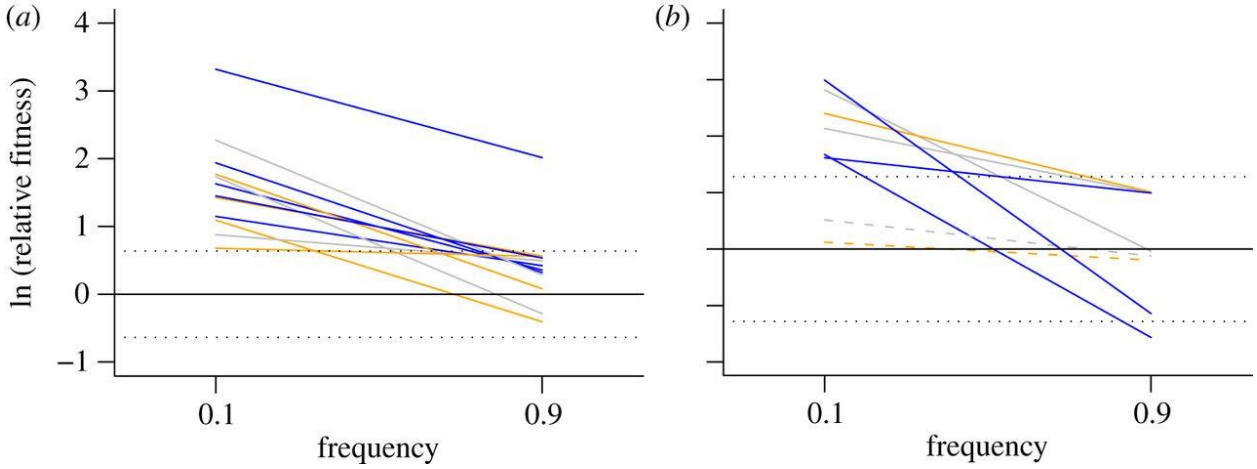

Figure 4. Frequency-dependent selection.

Lines correspond to independent lineages evolved in microcosms containing *Ps atrofaciens* (A) and *Pp ERA* (B) in which the specialists were still present after 20 transfers. We show the fitness of generalists relative to specialists measured in competition assays initiated at generalist frequencies of 0.1 and 0.9. Line colour and style corresponds to the representation in Figure 1. Dotted horizontal lines are the 95% confidence interval surrounding zero, calculated using the experiment-wide MSE (i.e. Fisher's Least Significant Difference).

REFERENCES

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends Ecol. Evol. 23:38–44.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410:463–6.

Doebeli, M. 2011. Adaptive diversification. Princeton University Press, Princeton, NJ.

Duffy, S., P. E. Turner, and C. L. Burch. 2006. Pleiotropic costs of niche expansion in the RNA bacteriophage φ6. Genetics 172:751–7.

Ferris, M. T. 2007. Evolution of the host range of the bacteriophage φ6. University of North Carolina at Chapel Hill.

Friesen, M. L., G. Saxer, M. Travisano, and M. Doebeli. 2004. Experimental evidence for sympatric ecological diversification due to frequency-dependent competition in *Escherichia coli*. Evolution. 58:245–60.

Mindich, L., J. F. Sinclair, D. Levine, and J. Cohen. 1976. Genetic studies of temperature-sensitive bacteriophage and nonsense mutants of φ6. Virology 223:218–223.

Pfennig, D. W., and M. McGee. 2010. Resource polyphenism increases species richness: a test of the hypothesis. Philos. Trans. R. Soc. London, Ser. B 365:577–591.

Rainey, P., A. Buckling, R. Kassen, and M. Travisano. 2000. The emergence and maintenance of diversity: insights from experimental bacterial populations. Trends Ecol. Evol. 15:243–247.

Rosenzweig, M. L. 1978. Competitive speciation. Biol. J. Linn. Soc. 10:275–289.

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford, U. K.

Simpson, G. G. 1953. The Major Features of Evolution. Columbia University Press, New York.

Smith, T. B., and S. Skúlason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu. Rev. Ecol. Syst. 27:111–133.

Vidaver, a K., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage φ6: a lipid-containing virus of *Pseudomonas phaseolicola*. J. Virol. 11:799–805.

Wilson, D. S., and M. Turelli. 1986. Stable underdominance and the evolutionary invasion of empty niches. Am. Nat. 127:835–850.

CHAPTER 3: REDUCED COMPETITION FACILITATES COEXISTENCE OF ALTERNATIVE RESOURCE-USE PHENOTYPES: AN EXPERIMENTAL TEST

Summary

Competition for resources is regarded as a key driver of adaptive diversification, but its role in the maintenance of diversity is less clear. Previously, we confirmed that competition among bacteriophage for bacterial hosts in microcosms containing both a standard laboratory host and a novel host triggered the evolution of a novel host-generalist phenotype. However, these new generalists tended to competitively exclude the ancestral host specialist. Coexistence between the two forms was rare, in part because generalists did not exhibit a fitness trade-off on the standard host. Here, we show that reducing the relative abundance of the novel host slowed the increase in frequency of the generalist phenotype, resulting in the emergence of trade-offs and of sustained coexistence. The slower ecological dynamics ensured the long-term stability of the coexistence by allowing sufficient time for adaptation of the specialists to generate a trade-off in host performance. Our results suggest that competition can indeed promote the origin and maintenance of resource use polymorphisms, even in the absence of antagonistic pleiotropy, as long as the ecological (inter-phenotype) dynamics are on a similar timescale to the evolutionary (intra-phenotype) dynamics.

Introduction

Resource competition has long been viewed as an important agent of diversifying selection (Schluter, 1994, Pfennig and Pfennig, 2012). When competition for a preferred resource is strong, competitively mediated selection will tend to favor individuals that can

exploit underutilized resources, even if these resources are novel, poor, or toxic (Bolnick, 2001). As a result, divergent selection drives phenotypes apart in niche space, thereby decreasing competition between them and allowing these phenotypes to coexist via negative frequency-dependent selection (Smith, 1962, Rosenzweig, 1978). Such resource polymorphisms (Smith and Skulason, 1996) have long fascinated evolutionary biologists, primarily because their evolution might represent a critical early stage in the origin of both novel resource-use traits and new species (Smith and Skulason, 1996, West-Eberhard, 2003, Pfennig and Pfennig, 2012). Thus, identifying the conditions under which resource polymorphism evolves is critical for understanding the origins and maintenance of biodiversity.

Competition does not always lead to diversification, and not all resource-use polymorphisms are evolutionarily stable. Competition must be sufficiently strong to give individuals that utilize relatively poor novel resources a selective advantage (Bailey et al., 2013), but not so strong that these individuals will competitively exclude the alternative resource-use phenotype(s). Coexistence typically results either from trade-offs, especially in systems at equilibrium, or niche partitioning, in systems with spatial (Rainey and Travisano, 1998) or temporal variation (Pfeiffer and Bonhoeffer, 2004, Helling et al., 1987, Rosenzweig et al., 1994, Turner et al., 1996, Treves et al., 1998, Rozen and Lenski, 2000). While some studies have directly shown that competition drives novel resource use, these studies often either end before examining the stability of the polymorphism (Bolnick, 2001) or end with no net increase in diversity (Bono et al., 2013). The conditions for coexistence are stringent (Rainey et al., 2000, Kassen, 2002), and only a few studies have shown that competition can promote both the origins *and* maintenance of resource polymorphism, even in relatively simple microbial microcosms (Tyerman et al., 2008, Rainey and Travisano, 1998).

Previously, we demonstrated that competition can favor the evolution of a novel resource-use morph in experimental populations of the bacteriophage φ6, but coexistence in these experiments was rare (Bono et al., 2013). Specifically, competition among these bacteriophage for bacterial hosts (a vital resource) in environments containing a 1:1 ratio of the standard laboratory host and a novel (non-permissive) host favored the evolution of a generalist that could utilize both the standard and novel hosts. However, these new generalist phage tended to competitively exclude the ancestral specialist phage. Therefore, coexistence between the two resource-use phenotypes was rare. These observations raise an important question: what ecological conditions promote the coexistence of alternative resource-use phenotypes in the same population; i.e., what conditions favor the *maintenance* of a resource polymorphism?

Here we explore the role of the ratio of standard to novel resources in the evolutionary stability of resource polymorphism. Using Resource-Ratio Theory (Tilman, 1980, 1982) as a guide, we predicted that increasing the ratio of standard:novel hosts would reduce the strength of selection favoring novel-host use but increase the probability of stable coexistence between alternative host-use phenotypes. Beginning with clonal populations of specialists that can only infect (utilize) the standard host, we monitored the evolution of generalists able to infect both standard and novel hosts over the course of 100 serial transfers (~300 generations) in liquid culture containing a 9:1 ratio of standard:novel hosts. As predicted, increasing the ratio of standard:novel hosts both slowed the emergence of host generalists and increased the proportion of lineages in which generalists coexisted stably with the ancestral specialist phage. We used a series of fitness assays in different environments to provide a mechanistic explanation for the difference in evolutionary trajectories and outcomes between the 9:1 and 1:1 host ratio environments.

Methods

Strains

The RNA bacteriophage φ6 used in this study is a laboratory strain descended from the original isolate (Vidaver et al., 1973). The bacterium *Pseudomonas syringae* pathovar phaseolicola strain HB10Y (Ferris et al., 2006, Duffy, 2005) served as the standard host and *P. pseudoalcaligenes* pathovar ERA (Ferris et al., 2006) as the novel host.

Culture Conditions

Details of diluting, filtering, culture and storage of phage and bacteria appear in (Ferris et al., 2006, Duffy, 2005). All phage and bacteria were grown in LC medium (5 g/liter yeast extract, 10 g/liter bactotryptone, 5 g/liter NaCl) at 25°C. Prior to the start of each serial transfer or assay, fresh hosts were grown to an OD_{600} corresponding to a density of 2×10^8 cells/mL (*Ps phaseolicola* OD_{600}=0.33 and *Pp ERA* OD_{600}=0.12).

We monitored the evolution of generalist phenotypes by plating phage population samples on lawns of 200 μL taken from a mixture of 1 mL of the standard host *Ps phaseolicola* and 10 μL of the novel host *Pp ERA*. Generalist phage that gained the ability to use the novel host produced clear plaques, whereas the specialist ancestor produced turbid plaques.

Competition Assays

We used competition experiments to assess the relative fitness of generalists and specialists in mixed and pure host environments. Generalist and specialist phage clones were isolated from populations of interest by plating a sample of the population on a mixed lawn of the standard and novel hosts and randomly harvesting a single generalist (clear plaque) and

specialist (turbid plaque). Generalist and specialist clones were then mixed in ratios that varied from 10%, 50%, or 90% generalists, depending on the experiment. These mixtures were incubated with either the standard host only or both hosts under conditions that exactly mimicked the evolution experiments. In particular, initial total host density was held at 2×10^8 cells/mL, initial total phage density at 10^8 phage/mL, and the resulting culture was incubated shaking for 6 hours at 25°C. The frequency of the generalist and specialist phage was determined by plating on mixed lawns at the beginning and end of each incubation. The fitness of the generalist relative to the specialist phage was calculated as $W=R_6/R_0$ where R_t is the ratio of generalists to specialists at time t hours.

Estimating Fitness Using Logistic Regression

In populations where generalists fixed (achieved a frequency $f_{gen}=1.0$), we used logistic regression to estimate fitness when common from the counts of generalists and specialists we obtained by sampling the evolving population at transfers in which $0.8<f_{gen}<0.97$. Logistic regression models are of the form:

$$logit(t)=ln\left(\frac{f_{gen}(t)}{1-f_{gen}(t)}\right)=\beta_0+\beta_1 t \qquad (1)$$

where β_1 is the expectation of lnW. We estimated $lnW=\beta_1$ using the function glm in R and obtained standard errors of the estimate using the function confint.

Adsorption Rate Assays

2000 phage were mixed with 1 mL of approximately 2×10^8 exponentially growing host cells and incubated shaking at 25°C. Initially, and after 40 minutes, 500 μL of this mixture was centrifuged for >1 minute at 6600 RPM to pellet the cells, and 200 μL of supernatant were plated on a lawn of the standard host *Ps phaseolicola* to obtain a count of the free phage. The adsorption rate constant was calculated as $k=-ln(P_{40}/P_0)/(N)$ where N = number of host cells, and P_t is the number of free phage at time t, determined by colony and plaque assays, respectively.

Statistical Analysis

Statistical analyses were conducted in R version 3.1.0. Linear mixed models were estimated using the lme function from the nlme package. Experimental lineage was treated as a random effect in all models. Competition treatment, population size, transfer, and initial generalist frequency were treated as fixed effects.

Experimental Design

Phage populations were evolved using serial transfer into fresh bacterial cultures containing a mixture of the standard laboratory host and a novel (non-permissive) host at a total density of 10^8 cells/mL (diagrammed in Figure 5). These evolution experiments were performed in the exact same manner as the populations under strong competition described in Bono et al. (2013) with one major exception: the host ratio was altered from a 1:1 ratio of standard to novel hosts to a 9:1 ratio (Figure 5). By increasing the density of standard hosts and decreasing the density of novel hosts, we reduced both intraspecific competition for the standard host and the

ecological opportunity represented by the novel host (ecological opportunity refers to "a wealth of evolutionarily accessible resources little used by competing taxa" (Schluter, 2000, p. 69). Replicate populations were founded by a single phage clone, i.e. phage harvested from a single isolated plaque. Individual populations were propagated by initiating each transfer at a multiplicity of infection (MOI or ratio of phage to hosts) of 0.1 and phage population sizes of N=10^5, 10^6 or 10^7 phage. Total culture volume was adjusted between 10 μL to 1 mL to achieve a constant host density and MOI across differences in phage population size. We varied population size in accordance with the methods described in Bono et al. (2013), although population size had no effect on the results and therefore population sizes were pooled there and here. Cultures were incubated shaking at 25°C for 6 hours and filtered to remove host cells, and a sample of 10^5, 10^6 or 10^7 of the resulting phage was used to initiate the next transfer cycle. We measured the total number of phage and the frequency of generalists at the end of every transfer.

Results

Evolution Experiment

Just as with the 1:1 host ratio (Figure 6A, warm colors, populations from (Bono et al., 2013)), φ6 populations under the 9:1 host ratio rapidly evolved new generalist morphs capable of infecting the novel host (Figure 6A, cool colors). However, the ultimate outcome of evolution depended on the ratio of standard to novel hosts. Under the 1:1 host ratio, generalists reached high frequencies in all population size treatments by transfer 20 (mean frequency ± s.e.m. for N=10^5: 0.968±0.014, N=10^6: 0.981±0.009, N=10^7: 0.949±0.030). Competitive exclusion was a likely outcome as over half (8/15) of all lineages achieved frequencies > 0.98 by transfer 20. In contrast, under the 9:1 host ratio generalists achieved lower frequencies at transfer 20 (mean

frequency ± s.e.m. for $N=10^5$: 0.257±0.062, $N=10^6$: 0.431±0.037, $N=10^7$: 0.561±0.070), and coexisted with specialists for the duration of our experiment in all replicate lineages. Under the 9:1 host ratio, generalists never exceeded a frequency above 0.80 in any transfer.

To determine the role of host ratio (i.e., of ecology) in the observed coexistence, we examined the stability of the coexistence after only 20 transfers, a time point sufficiently early that we do not expect significant evolutionary differences to have accumulated between populations evolved in different host ratios. We examined stability of coexistence by measuring the relative fitness of generalist phage when the generalist is common (Figure 6B). The stability of the observed coexistence is confirmed only if generalists exhibit a disadvantage ($lnW<0$) when common. As expected from the difference in their frequency trajectories, generalists in populations evolved under the 9:1 host ratio exhibited significantly lower relative fitness when common than generalists in populations evolved under 1:1 host ratio ($t=2.879$, $df=17.732$, $p=0.0101$ by a 2-sided t-test). Furthermore, generalists evolved in the 1:1 host ratio exhibited significant advantages when common (mean $lnW=-0.467$, $t=-2.958$, $df=5$, $p=0.0158$ by a 1-sided t-test), whereas generalists evolved in the 9:1 host ratio did not (mean $lnW=0.269$, $t=1.338$, $df=14$, $p=0.899$). These data confirm that the coexistence of generalists and specialists was stable in the 9:1 host ratio populations even at this very early time point. Analogous data collected at later time points from the 9:1 host ratio populations confirmed that the observed coexistence remained stable throughout the 100 transfer experiments (Figure 7).

Mechanistic Explanations of the Difference in Competitive Outcome.

Explanation 1. Ecology.

We investigated the proximate mechanisms responsible for the coexistence of generalist and specialist phage in the 9:1 host ratio. Starting with an ecology-only mechanism, we assessed whether the difference in host ratio, by itself, could explain the observed differences in coexistence between host ratio treatments. As above, we assayed generalist fitness when common in populations evolved for 20 and for 100 transfers in the 9:1 host ratio, this time altering the host ratio to 1:1 for a single transfer. Recall that populations evolved in the 1:1 host ratio did not tend to exhibit a disadvantage when common in the 1:1 host ratio environment. By contrast, populations evolved in the 9:1 host ratio tended to exhibit a disadvantage when common both in the 9:1 host ratio that they experienced and in the 1:1 host ratio that they did not experience (Figure 8, transfer 20: grand mean $lnW=-0.361$, $t=-2.34$, $df=5$, $p=0.0333$; transfer 100: grand mean $lnW-0.58$, $t=-1.84$, $df=2$, $p=0.104$ by 1-tailed t-tests conducted on the population means). In fact, the average lnW of populations evolved in the 9:1 host ratio did not depend significantly on the host ratio used to assay fitness (Figure 8, transfer 20: $F_{1,52} = 0.490$, $p = 0.487$; transfer 100: $F_{1,26} = 0.142$, $p = 0.710$, statistical comparisons from mixed-effects linear models). These data indicate that host ratio (i.e., "ecology"), alone, could not explain the difference in competitive outcomes between our host ratio treatments.

Explanation 2. Genetic Basis of Adaptation.

Next, we investigated an evolutionary explanation for the difference between competition treatments. We determined if the initial genetic basis of adaptation in the generalists differed between host ratios by comparing the host adsorption rate (the primary target of selection for

host range expansion (Ferris et al., 2006, Bono et al., 2013)) of generalists evolved under both host ratios (Figure 9). We measured adsorption rates of generalist clones isolated randomly from all 6 transfer-20 9:1 host ratio populations, from 6 randomly chosen transfer-20 1:1 host ratio populations, and from all 3 transfer-100 9:1 host ratio populations. In Figure 9A we compare adsorption rates of the transfer-20 generalists between the host ratio treatments. Comparing the 1:1 and 9:1 host ratio treatments at transfer 20, we observed no significant difference in adsorption rates to either the standard host ($F_{1,10}$=0.276 , p=0.6108) or the novel host ($F_{1,10}$=1.864 , p=0.2021), suggesting similar initial genetic bases of host-range expansion regardless of host ratio. Comparing the transfer-20 and transfer-100 phage from the 9:1 host ratio treatment (Figure 9B), we observed an increase in adsorption to the standard host ($F_{1,7}$=9.726 , p=0.0169) but not to the novel host ($F_{1,7}$=2.97326 , p=0.1283). This higher adsorption rate of the evolved generalists to the standard host is unlikely to have contributed to coexistence with the specialist, because it made the generalist a stronger competitor for the sole host resource of the specialist. Thus, these data indicate that "evolution", alone, could not explain the difference in competitive outcomes between our host ratio treatments.

Explanation 3. Ecology Enabled Evolution.

Finally, we turned to an explanation for the differences in coexistence that required both differences in ecology and evolution between treatments. One such explanation is suggested by the frequency dynamics shown in Figure 6. As can be seen, the reduction in the ratio of novel:standard hosts slowed the increase in frequency of generalist phage, i.e. the ecological dynamics. These slower ecological dynamics appear to have allowed time for the specialists to

adapt. The initial increase in generalist frequency is followed by a decline between around transfers 30 to 50, suggestive of an adaptive sweep (Helling et al., 1987) within the specialists.

To confirm adaptation of the specialists over the course of the 9:1 host ratio experiment, we compared the relative fitness of evolved and ancestral specialist phage when grown on the standard host only, using an evolved generalist as a common competitor. We repeated this comparison for evolved phages isolated from transfer 20 and transfer 100. Three important observations can be made from these data (Figure 6). First, evolved specialists exhibited higher fitness than the ancestral specialist at transfer 100 (mean fitness gain ± *s.e.m.*=0.454±0.125, t=3.628, df=26, p=0.0012), confirming adaptation by the specialists over the course of the experiment. None of this adaptation was apparent at transfer 20 (mean fitness gain ±*s.e.m.*=−0.159±0.155, t=−1.025, df=26, p=0.3147). Second, *evolved* specialists exhibited a fitness advantage ($lnW > 0$) over evolved generalists at both transfers 20 and 100 (Figure 10), confirming the presence of a host-use trade-off at both early and late transfers. Third, the *ancestral* specialist exhibited a fitness advantage over evolved generalists in all 3 populations at transfer 20 (Figure 10A), indicating that antagonistic pleiotropy in the generalists created the trade-off at this early time point. By transfer 100, the advantage of the ancestral specialist over the evolved generalists disappeared from 2 of the 3 populations (Figure 10B), suggesting that antagonistic pleiotropy was ameliorated in these populations by the end of the experiment. Thus, these data suggest that differences between treatments in both ecology and evolution were required to explain the difference in competitive outcomes between our host ratio treatments.

Discussion

By altering resource ratio, we investigated the effects of resource competition and ecological opportunity on the origin and maintenance of resource-use polymorphism. We found

that a skewed (9:1) ratio of standard:novel hosts enabled coexistence of alternative host-use phenotypes more often than a 1:1 host ratio. Skewing the host ratio slowed down the ecological dynamics between the two phenotypes (i.e., “inter-phenotype” dynamics), providing sufficient time for the specialists to adapt before the generalists swept through the experimental populations. This adaptation by the specialists (i.e., “intra-phenotype” dynamics) appeared to be essential for coexistence, explaining both the difference in coexistence between 9:1 and 1:1 host ratio experiments and the ability of populations evolved in the 9:1 host ratio to sustain coexistence even when shifted into the 1:1 host ratio environment. This last observation indicates that, although the host ratio required for the origin of a stable resource polymorphism was stringent, once the stable polymorphism was established, coexistence was maintained across a wide range of host ratios. This relaxation in the stringency of ecological requirements for coexistence over time seems likely to characterize any population in which both alternative resource-use morphs are adapting to their changing environment.

Resource-ratio theory predicts that the outcome of competition—i.e., competitive exclusion versus coexistence—depends on both the ratio of limiting resources and the strength of trade-offs in performance on the two resources (Tilman, 1980, Miller et al., 2005, Tilman, 1982). In our experiments, there was temporal heterogeneity in the densities of both standard and novel hosts over the course of the 6 hour incubation between serial transfers. The initial host ratio and the strength of performance trade-offs determined quantitatively how the host density dynamics translated into concurrent dynamics in the fitness of generalists relative to specialists. When generalists were rare, the fitness of generalists relative to specialists grew as the incubation proceeded because standard hosts became scarce and specialists ran out of resources. Because this advantage of the generalist when rare was large, it did not depend strongly on either host

ratio or the strength of performance trade-offs. When generalists were common, they no longer experienced a period when novel hosts were abundant but standard hosts were scarce. In this scenario, the major advantage of being a generalist was a reduction in the time spent adsorbing to (i.e., searching for) a host, and the strength of this advantage depended on host ratio. The disadvantage of being a generalist arose from performance trade-offs. Our combined results suggest that generalists experienced a net disadvantage when common in the 9:1 host ratio experiments both because their search time advantage was weaker and because adaptation by the specialist ensured that performance trade-offs were stronger than in the 1:1 host ratio experiments.

We found that host-use polymorphisms evolved in a 9:1 host ratio were stable in a 1:1 host ratio as early as transfer 20, even though polymorphisms evolved in the 1:1 host ratio were not. Conceptually, this difference in coexistence stability in the 1:1 host ratio environment could have resulted from differences in search time or in the strength of performance trade-offs between populations evolved in the 9:1 and 1:1 host ratio treatments. We observed no difference between the treatments in adsorption rate to either the standard or novel hosts, ruling out differences in search time. We also observed no adaptation by the specialist at this early transfer, ruling out the possibility that infecting a single host lead the specialist to adapt to the standard host faster. The only explanation that remains is that trade-offs at this early time point arose directly via antagonistic pleiotropy–a negative genetic correlation between performance on the two host resources (Elena and Lenski, 2003, Lynch and Gabriel, 1987, Levins, 1968, Rausher, 1984). Although generalists evolved using a 9:1 host ratio did not exhibit a reduced adsorption rate on the standard host, they did exhibit an overall decrease in performance on the standard host at transfer 20. We observed no such decrease in performance by generalists evolved in the

1:1 host ratio (Bono et al., 2013). A possible explanation for these differences is that the mutations responsible for the initial host range expansions in the 9:1 host ratio were more often characterized by antagonistic pleiotropy than in the 1:1 host ratio. We found that in both environments the initial host range expansions were characterized by antagonistic pleiotropy, but this antagonistic pleiotropy was ameliorated by subsequent adaptation of the generalists. The slower dynamics in the 9:1 host ratio allowed us to observe both the existence of antagonistic pleiotropy at transfer 20 and its amelioration by transfer 100. We posit that the faster dynamics in the 1:1 host ratio ensured that the initial antagonistic pleiotropy had already been ameliorated by transfer 20. This explanation is the only one consistent with all of our data. If generalists did have a greater opportunity for adaptation in the 1:1 host ratio environment during the first 20 transfers, it strengthens the argument that ecological (inter-phenotype) dynamics affect the outcome of competition via their influence on the evolutionary (intra-phenotype) dynamics.

Our data therefore suggest that resource polymorphism can arise, and be maintained stably, even in systems in which antagonistic pleiotropy is not universal. One of the major challenges we had in designing our experiments and interpreting our experimental outcomes was that our conceptual expectations were derived from models in which antagonistic pleiotropy is guaranteed (e.g. mutations affecting beak size in finches faced with small and large seeds universally exhibit antagonistic pleiotropy (Dieckmann and Doebeli, 1999)). Our data demonstrate that competition can drive the origin and maintenance of resource polymorphism, even in the absence of antagonistic pleiotropy, as long as the ecological (inter-phenotype) dynamics are on a similar timescale to the evolutionary (intra-phenotype) dynamics. The framework for investigating the consequences of this concordance of timescales seems to exist in the study of evolutionary rescue, the scenario in which populations must win the evolutionary

"race against extinction" following an ecological shift (Carlson et al., 2014). Although the theoretical and empirical focus has been on evolutionary rescue following a shift in environment (Carlson et al., 2014, Osmond and de Mazancourt, 2012), the idea should be extendable to evolutionary rescue following introduction of a competitor. Our data indicate that, while competition is a necessary condition for the maintenance of diversity, evolutionary rescue may also play a major role.

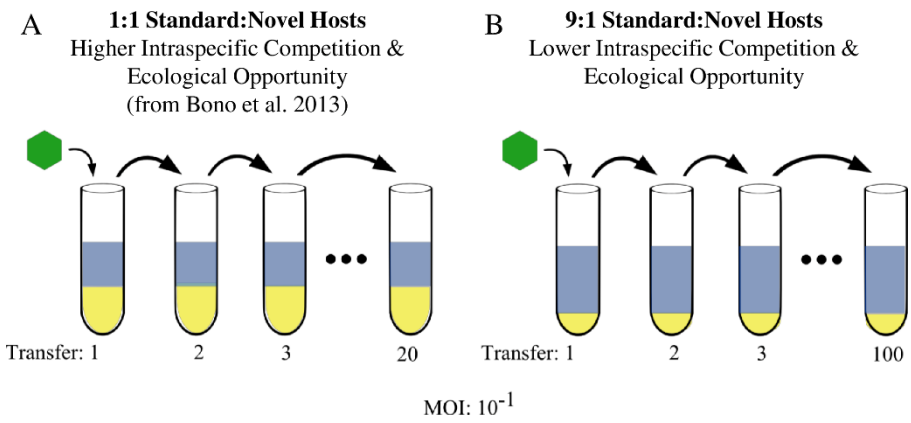

Figure 5. Experimental design.

Our experiment compared the evolution and maintenance of host-use polymorphism during serial transfer into fresh cultures containing different ratios of the standard and novel hosts. Cultures contained either a 1:1 ratio (A) or a 9:1 ratio (B) of standard (blue) to novel (yellow) hosts. Although the phage population bottleneck at the start of each transfer varied from N=10^5 to 10^7, total culture volume was adjusted accordingly so that all transfers were initiated at MOI = 10^{-1}. Populations were evolved for 20 transfers under the 1:1 host ratio or 100 transfers under the 9:1 host ratio

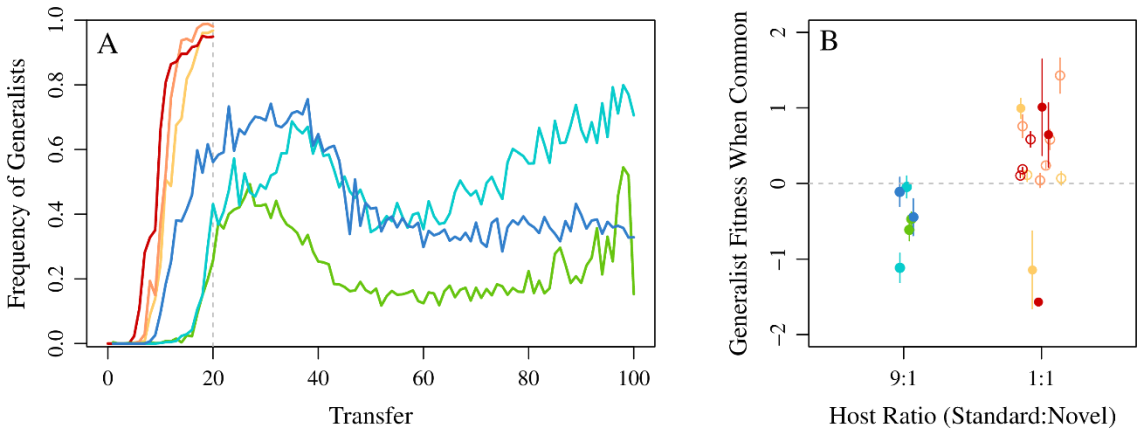

Figure 6. Generalist frequency over time and coexistence.

In both panels color distinguishes populations evolved under 9:1 host ratio (cool colors; transfer population sizes N=10^5: green, 10^6: light blue, 10^7: blue) or 1:1 host ratio (warm colors; transfer population sizes N=10^5: yellow, 10^6: orange, 10^7: red). (A) Frequency of generalists over time, averaged across replicate populations for each host ratio by population size treatment combination. Each population size treatment was conducted with 5-fold replication under the 1:1 host ratio and with 2-fold replication under the 9:1 host ratio through transfer 20. After transfer 20, each 9:1 host ratio line corresponds to a single replicate population. (B) Mean ln(fitness) ± s.e.m. of generalists relative to specialists for each replicate population at transfer 20, assayed at a high initial generalist frequency of approximately 0.9. Open and closed circles represent populations in which specialists were absent (i.e. had gone extinct) or present at transfer 20, respectively.

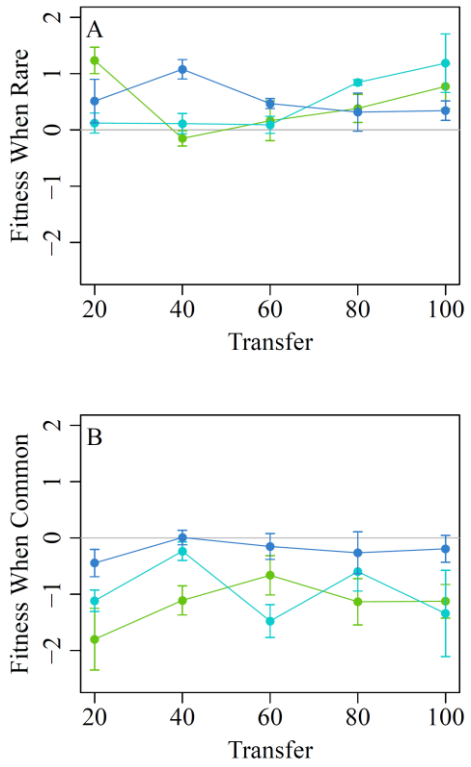

Figure 7. Frequency-dependent selection over time.

(A) The fitness of the generalists relative to the specialists when the generalists are initially rare (f_{gen} ~ 0.1), measured at transfers 20, 40, 60, 80, and 100. (B) The relative fitness measured repeated when the generalists are initially common (f_{gen} ~ 0.9). Colors are the same as in Figure 6.

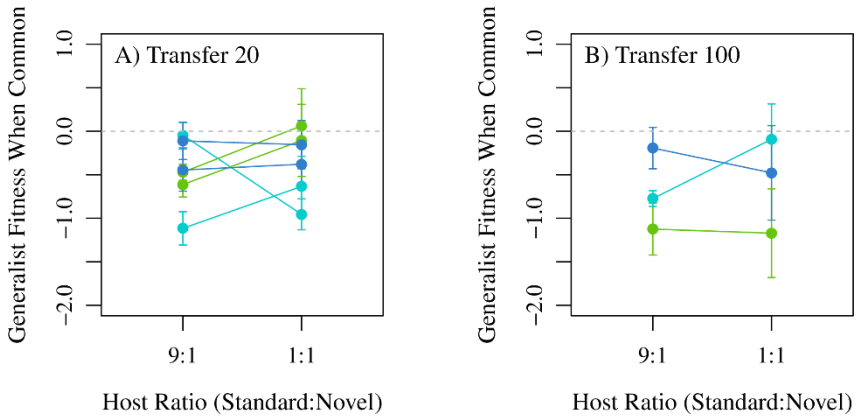

Figure 8. Relative fitness of evolved generalist in alternative competitive environments. Pairs of generalist and specialist phage clones were randomly isolated from each transfer-20 and transfer-100 population and subjected to competition assays in both the 9:1 host ratio to which they were adapted and the 1:1 host ratio. Data are means ± s.e.m. of the ln(fitness) of the generalist relative to specialist in assays initiated at generalist frequencies around 0.9. Colors are the same as in Figure 6.

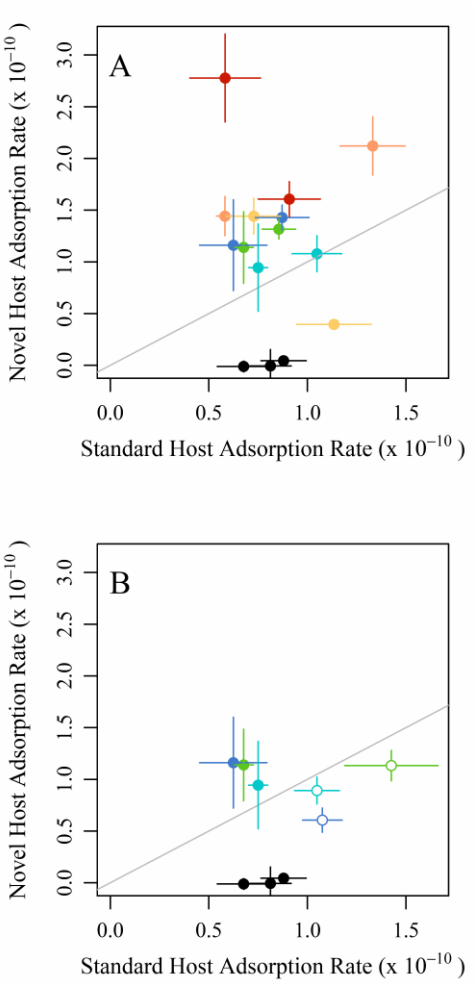

Figure 9. Adsorption rates of evolved generalists.

Points ± 95% CI show phage adsorption rates on the standard and novel hosts; color corresponds to the representation in Figure 6. (A) compares evolved generalist phage isolated after 20 transfers under the 1:1 host ratio (warm colors) versus the 9:1 host ratio (cool colors) competition. (B) compares generalist phage isolated after 20 (closed circles) or 100 (open circles) of evolution under the 9:1 host ratio. Ancestral phage are shown in black for reference. The gray line corresponds to equal adsorption to both hosts.

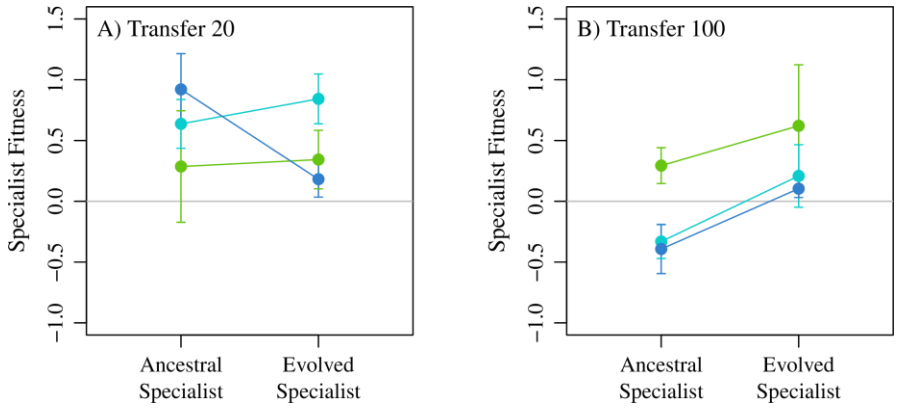

Figure 10. Adaptation to the standard host.

The fitness of evolved and ancestral specialist clones was determined from competition assays on the standard host only, using an evolved generalist clone as a common competitor. The evolved generalist and specialist clones were isolated randomly from either the transfer-20 populations (A) or the transfer-100 populations (B). Data are mean *lnW* of specialists relative to generalists ± 90% confidence intervals. Error bars that do not span zero indicate a significant fitness advantage of the specialist over the generalist; color corresponds to the representation in Figure 6.

REFERENCES

Bailey, S. F., J. R. Dettman, P. B. Rainey, and R. Kassen. 2013. Competition both drives and impedes diversification in a model adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences*, 280(1766):20131253–20131253.

Bolnick, D. I. 2001. *Nature*, 410(6827):463–466.

Bono, L. M., C. L. Gensel, D. W. Pfennig, and C. L. Burch. 2013. Competition and the origins of novelty: experimental evolution of niche-width expansion in a virus. *Biology Letters*, 9(1):20120616–20120616.

Carlson, S. M., C. J. Cunningham, and P. A. Westley. 2014. Evolutionary rescue in a changing world. *Trends in Ecology & Evolution*, 29(9):521–530.

Dieckmann, U. and M. Doebeli. 1999. *Nature*, 400(6742):354–357.

Duffy, S. 2005. Pleiotropic Costs of Niche Expansion in the RNA Bacteriophage 6. *Genetics*, 172(2):751–757.

Elena, S. F. and R. E. Lenski. 2003. Microbial genetics: Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nat Rev Genet*, 4(6):457–469.

Ferris, M. T., P. Joyce, and C. L. Burch. 2006. High Frequency of Mutations That Expand the Host Range of an RNA Virus. *Genetics*, 176(2):1013–1022.

Helling, R., C. Vargas, and J. Adams. 1987. Evolution of Escherichia coli during growth in a constant environment. *Genetics*, 116:349–58.

Kassen, R. 2002. The experimental evolution of specialists generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, 15(2):173–190.

Levins, R. 1968. Ecological Engineering: Theory and Technology:Ecology and Resource Management: A Quantitative Approach. Kenneth E. F. Watt. *The Quarterly Review of Biology*, 43(3):301.

Lynch, M. and W. Gabriel. 1987. Environmental Tolerance. *The American Naturalist*, 129(2):283.

Miller, T. E., J. H. Burns, P. Munguia, E. L. Walters, J. M. Kneitel, P. M. Richards, N. Mouquet, and H. L. Buckley. 2005. A Critical Review of Twenty Years' Use of the Resource-Ratio Theory. *The American Naturalist*, 165(4):439–448.

Osmond, M. M. and C. de Mazancourt. 2012. How competition affects evolutionary rescue. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1610):20120085–20120085.

Pfeiffer, T. and S. Bonhoeffer. 2004. Evolution of Cross-Feeding in Microbial Populations. *Am Nat*, 163(6):E126–E135.

Pfennig, D. W. and K. S. Pfennig. 2012. *Evolution's wedge: competition and the origins of diversity*, number 12. Univ of California Press.

Rainey, P. B., A. Buckling, R. Kassen, and M. Travisano. 2000. The emergence and maintenance of diversity: insights from experimental bacterial populations. *Trends in Ecology & Evolution*, 15(6):243–247.

Rainey, P. B. and M. Travisano. 1998. *Nature*, 394(6688):69–72.

Rausher, M. D. 1984. Tradeoffs in performance on different hosts: evidence from within- and between-site variation in the beetle *Deloyala guttata*. *Evolution*, 38(3):582.

Rosenzweig, M. L. 1978. Competitive speciation. *Biological Journal of the Linnean Society*, 10(3):275–289.

Rosenzweig, R. F., R. Sharp, D. S. Treves, and J. Adams. 1994. Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. *Genetics*, 137(4):903–917.

Rozen, D. E. and R. E. Lenski. 2000. Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism. *Am Nat*, 155(1):24–35.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science*, 266(5186):798–801.

Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford University Press.

Smith, J. M. 1962. Disruptive selection polymorphism and sympatric speciation. *Nature*, 195(4836):60–62.

Smith, T. B. and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.*, 27(1):111–133.

Tilman, D. 1980. Resources: A graphical-mechanistic approach to competition and predation. *The American Naturalist*, 116(3):362.

Tilman, D. 1982. Resource competition and community structure. *Monogr Popul Biol*, 17:1–296.

Treves, D. S., S. Manning, and J. Adams. 1998. Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of *Escherichia coli*. *Molecular Biology and Evolution*, 15(7):789–797.

Turner, P. E., V. Souza, and R. E. Lenski. 1996. Tests of ecological mechanisms promoting the stable coexistence of Ttwo bacterial genotypes. *Ecology*, 77(7):2119.

Tyerman, J. G., M. Bertrand, C. C. Spencer, and M. Doebeli. 2008. Experimental demonstration of ecological character displacement. *BMC Evol Biol*, 8(1):34.

Vidaver, A., R. Koski, and E. J. Van. 1973. Bacteriophage phi6: a lipid-containing virus of *Pseudomonas phaseolicola*. *J Virol*, 11:799–805.

West-Eberhard, M. J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press.

CHAPTER 4: ADAPTATION TO A NOVEL RESOURCE IN SYMPATRY WITH A COMPETITOR VERSUS ALLOPATRY: AN EXPERIMENTAL TEST IN A VIRUS

Summary

Competition for resources has long been thought to be a key driver of diversification. In a previous study, we found that competition alone promoted resource (host) range expansion in the bacteriophage φ6. Generalists, able to use both the original, standard host and a novel host, rapidly increased when the supply of host cells were restricted. These generalists expanded their host range without diminishing their fitness on the standard host. Using a pair generalists and specialists that coexist via niche partitioning, we initiated a new evolution experiment to test if the presence of a competitor acts a wedge between the phenotypes, driving the generalists towards specialization on the novel host. Specifically, we evolved the generalist with a competitor (specialist) in sympatry with both hosts and alone in allopatry with only a novel host as a control. Generalists evolved in both treatments maintained the same host range, but allopatric generalists used the standard host significantly less than the sympatric generalists. Rather than diverging from the specialists, sympatric generalists actually increased in their adsorption rate to the standard host. We discuss these outcomes in the context of previous studies that documented a high variation in pleiotropic effects among mutations that expand host range in φ6. We found that allopatric generalists showed decreased performance on the standard host, because in the absence of selection on the standard host, the average pleiotropic effect of host range expansion is antagonistic (negative) and mutations with negative effects on standard host performance were selectively neutral in allopatry. Sympatric generalists showed increased

performance on the standard host, because selection favored rare host range mutations that exhibit positive pleiotropy where both hosts were present. Although the conceptual focus in the field has been on the role of gene flow in slowing divergence in sympatry, these results suggest that divergence can be also be prevented when phenotypes adapt in the absence antagonistic pleiotropy.

Introduction

Ecology has long been viewed to play a significant role in promoting biological diversity, and Darwin (1859) proposed that natural selection promotes divergence and potentially the formation of new species. Theory holds that in a population experiencing intense competition, frequency-dependent selection will favor individuals that can exploit an underutilized resource—even if this resource is novel, of lower quality, or toxic—because these individuals should benefit from decreased competition (Rosenzweig, 1978; Wilson and Turelli, 1986). Such novel resource use can lead to a shift or expansion of a species' niche (Bolnick, 2001). Such novel resource use is posited to be accompanied by a trade-off, notably antagonistic pleiotropy, on the standard host. Eventually, this could possibly lead to the evolution of a resource-use polymorphism in which alternative phenotypes with different resource use occur in a single population (Smith and Skulason, 1996; Pfennig and Pfennig, 2012; Pfennig and Murphy, 2003; Bono et al., 2013).

The evolution of a resource-use polymorphism may also represent a critical, early stage in the sympatric speciation process (West-Eberhard, 2003). Specifically, as alternative resource-use phenotypes come to occupy separate niches, selection should favor traits that improve each phenotype's ability to survive and reproduce in its particular niche. As alternative phenotypes in a resource polymorphism become more distinct—ecologically, phenotypically and genetically—

they may eventually become reproductively isolated from each other through ecological speciation (Rundle and Nosil, 2005). However, there are a few major obstacles, particularly gene flow, that could prevent competition from acting as a wedge to separate alternative phenotypes apart in niche space.

Whether a phenotype adapts alone in allopatry or in the presence of another phenotype in sympatry is thought to have a major effect on divergence under the scenario described above, because divergence in sympatry is theoretically difficult. While gene flow has been hypothesized to disrupt the process of divergence via hybridization of the alternative phenotypes (Mayr, 1963; Coyne et al., 2004), competition has hypothesized to act as a wedge to separate the phenotypes apart in niche space, reducing gene flow between them (Dieckmann and Doebeli, 1999; Mallet, 2008; Nosil 2012, 2008; Flaxman et al., 2013; Via, 2001; Pfennig and Pfennig, 2012). Despite the conceptual focus on the role of gene flow in slowing divergence in sympatry, our previous results (Bono et al., 2013 and Chapter 3) suggest that another property of sympatric speciation–the presence of multiple resources—may also be sufficient to slow divergence *even in the absence of antagonistic pleiotropy*.

Few studies have taken a direct experimental approach to establish a causal link between the presence of a competitor phenotype and divergence in resource use. Our study sought to fill this gap using evolution experiments in the RNA bacteriophage (bacteria-infecting virus) φ6. This study builds on previous work in φ6 demonstrating that competition for resources drives novel resource (host) use (Bono et al., 2013). Here, we evolve the generalist alone in allopatry with just the novel host or in the presence of a competitor (the specialist) in sympatry with both hosts. We used the sole pair of coexisting generalists and specialists, which had already diverged in host use (Bono et al., 2013), to found these populations. By comparing the degree of novel

host specialization, we test if generalists diverged in host use more when evolved alone in allopatry (the null hypothesis) or if competition acts as wedge to drive the phenotypes apart in niche space with generalists evolving toward standard host specialization.

Methods

Strains

The RNA bacteriophage φ6 used in this study is a laboratory strain descended from the original isolate (Vidaver et al., 1973). The bacterium *Pseudomonas syringae* pathovar phaseolicola strain HB10Y (Ferris et al., 2006; Duffy et al., 2006) served as the standard host and *P. pseudoalcaligenes* pathovar ERA (Ferris et al., 2006) as the novel host. φ6 is an appropriate model system for studying the consequences of gene flow, because it can exchange genomic segments via reassortment during coinfection (Chao, 1988, 1990, 1992; Turner and Chao, 1999).

Culture Conditions

Details of diluting, filtering, culture and storage of phage and bacteria appear in Ferris et al., (2006) and Duffy et al. (2006). All phage and bacteria were grown in LC medium (5 g/liter yeast extract, 10 g/liter bactotryptone, 5 g/liter NaCl) at 25°C. Prior to the start of each serial transfer or assay, hosts were grown to an OD_{600} corresponding to a density of 2×10^8 cells/mL (*Ps phaseolicola* OD_{600}=0.33 and *Pp ERA* OD_{600}=0.12).

We monitored the evolution of generalist phenotypes by plating phage population samples on lawns of 200 μL taken from a mixture of 1 mL of the standard host *Ps phaseolicola*

and 10 μL of the novel host *Pp ERA*. Generalist phage that gained the ability to use the novel host produced clear plaques, whereas the specialists produced turbid plaques.

Evolution Experiment

Our experimental design is diagrammed in Figure 11. Populations were founded with a single generalist and a single specialist isolate from the end of the evolution experiment in Bono et al., (2013), using the sole pair of phenotypes that coexisted after 20 transfers, and we evolved φ6 via serial transfer into fresh bacterial cultures containing 10^8 cells/mL (Figure 5). For sympatric populations, we initiated the evolution with 1:1 ratio of specialists and generalists so that neither had a demographic advantage on a 1:1 ratio of standard and novel hosts. For allopatric populations, generalists alone were incubated on only *Pp ERA*. At the start of a transfer, cultures of exponentially growing bacteria were inoculated with 10^6 phage, incubated shaking to ensure spatial homogeneity at 25°C for 6 hours and filtered to remove host cells. Phage density was determined by plating a sample. A volume corresponding to 10^6 phage was used to initiate the next transfer and the remainder of the sample was archived at -20°C for future analysis. This protocol was repeated for 100 transfers. We evolved 5 replicate populations for both sympatric and allopatric treatments.

Detection of Novel Host Specialist

Because we were unable to detect novel host specialists on mixed host lawn plates, we employed an alternative method to test if a novel host specialist reached ≥ 1% of the population. We plated a sample on a novel host lawn. Plaques produced on the novel host are either generalists or novel host specialists. To differentiate between the two, we transferred 100 of those plaques to a standard host lawn via sterile toothpick (and then immediately transferred to a

novel host lawn as confirmation of successful transfer): novel host specialists only produce plaques on the novel host while generalists produce plaques on both.

Adsorption Rate Assays

2000 phage were mixed with 1 mL of approximately 2×10^8 exponentially growing host cells and incubated shaking at 25°C. Initially, and after 40 minutes, 500 μL of this mixture was centrifuged for >1 minute at 6600 RPM to pellet the cells, and 200 μL of supernatant were plated on a lawn of the standard host to obtain a count of the free phage. The adsorption rate constant was calculated as $k=-ln(P_{40}/P_0)/(40N)$ where N = number of host cells, and P_t is the number of free phage at time t, determined by colony and plaque assays, respectively.

Statistical Analysis

Statistical analyses were conducted in R version 3.1.2. Linear mixed models were estimated using the lme function from the nlme package. Population was treated as a random effect in all models. Treatment (sympatry or allopatry), phenotype, and date on which the assay was performed were treated as fixed effects. Type III tests of fixed effects are reported in order to account for the effect of the date on which the assay was performed.

Results

Evolution Experiment

We monitored the population dynamics by tracking the frequencies of the standard host specialist and generalist phenotypes at the end of each transfer. Since competitive exclusion was a concern, we confirmed that generalists coexisted with their specialist competitors in all sympatric populations. The average frequency of the generalists ranged between 0.349-0.609.

We tested if a phenotype evolved to restrict its host range to solely the novel host, becoming a novel host specialist and thus eliminating the possibility of gene flow between with the standard host specialist in a situation analogous to speciation via magic trait (Servedio et al., 2011). Although we checked every 10 transfers, we were unable to detect that a novel host specialist arose to 1% in any sympatric or allopatric populations.

Unexpectedly, a novel generalist phenotype, forming a bull's-eye plaque (Figure 12A), arose in 2 of the 5 sympatric populations (Figure 12B) but 0 of the 5 allopatric populations. This phenotype produced plaques on both the standard and novel hosts, confirming its host range as a generalist. We tracked it within those populations once it reached 1% of the population.

Adsorption rate

We assessed how generalists evolved in sympatry and allopatry by comparing the adsorption rates on the competitive treatment of the generalist (ancestor, sympatry, or allopatry) to both the standard and novel hosts separately (Figure 13). Initially, we restricted our comparison of generalists to just the clear phenotype, because we wanted to compared the generalists that evolved from a common ancestor and were uncertain if the bull's-eye generalist evolved from a specialist or generalist. The competitive treatment had a significant effect on standard host adsorption ($F_{2,8}$ = 14.860, p = 0.0148) as well as date ($F_{7,83}$ = 3.806, p = 0.0012). Sympatric generalists adsorb $3.214\times10^{-11}\pm8.339\times10^{-12}$ (t_8 = 3.855, p = 0.0048) faster to the standard host than allopatric generalists, so while the sympatric generalists increase in adsorption rate to the standard host, the allopatric generalists decrease in standard host adsorption. However, there is no significant difference in adsorption rate between the ancestor and allopatric generalists $2.291\times10^{-11}\pm2.898\times10^{-11}$ (t_8 = 0.791, p = 0.4519) or sympatric generalists

$-9.260\times10^{-12}\pm2.898\times10^{-11}$ (t_8 = -0.320, p = 0.7575) on the standard host. (The standard error of the ancestor is far larger than either the sympatric or allopatric generalists, because only 10 observations were measured for the ancestor while sympatric and allopatric generalists each had 50 (5 populations x 10 replicates)). However, the mean of 4/5 allopatric populations was less than the mean of the ancestor, and the mean of 5/5 sympatric populations was greater than the mean of the mean of the ancestor. There is no significant difference in adsorption rate to the novel host for competitive treatment ($F_{1,8}$ = 0.918, p = 0.3661), but date was again significant ($F_{7,83}$ = 8.026, p < 0.0001).

We then compared the adsorption rates of the clear and bull's-eye generalist phenotypes for the two populations in which the bull's-eye phenotype evolved. They differed significantly on the novel host (phenotype: $F_{1,29}$ = 83.040, p < 0.0001, date: $F_{8,29}$ = 2.243, p = 0.0532) but not the standard host (phenotype: $F_{1,29}$ = 0.146, p = 0.7051, date: $F_{8,29}$ = 1.444, p = 0.2210). The bull's-eye phenotype displays a strong specificity for the standard host, meaning they adsorb the standard host far faster than the novel host. The clear generalists from the same populations, on the other hand, have a relative parity in host adsorption.

Discussion

We demonstrated that a generalist phenotype adapts to a novel host differently when evolved alone in allopatry or with a competitor in sympatry. We predicted that generalists would diverge from specialists in both allopatric and sympatric treatments but that competition would act as a wedge, accelerating the pace of divergence in sympatry. Allopatric generalists decreased in adsorption to the standard host as expected. However, we found that sympatric generalists, instead, increased adsorption to the standard host, becoming more similar to specialists. Recall

that the sympatric and allopatric treatments differed by both the presence of a competitor (and potentially gene flow with it) but also in the hosts that were present: the standard host was necessary for the specialist in sympatry. This suggests that the availability of the standard host, despite competition for it, presented a benefit to the generalists in sympatry and that selection acted to minimize antagonistic pleiotropy in standard host adsorption.

Since competition failed to act as a wedge, let us consider the mechanisms that can lead the alternative phenotypes to converge in host use in sympatry. First, this result could arise from gene flow between generalists and specialists. Segmented viruses, such as φ6, can exchange genes via reassortment, a process that occurs when two virions co-infect the same cell and produce hybrid offspring containing a mix of the parental genome segments (Delbrück and Bailey 1946; Malmberg, 1977). This process can only occur when a generalist and specialist co-infect a standard host cell. Even though the generalist had high novel host specificity initially, reassortment with the specialist may have introduced alleles adapted to the standard host. As the generalist adapts to the standard host and therefore infects it more often, the probability of gene flow with the specialist increases via positive feedback.

Although gene flow is often cited as a potentially insurmountable obstacle to the divergence, host environment may have had greater influence in our experiments, particularly as gene flow was rare. Adaptation to a novel host is specific to host environment; for instance, vesicular stomatitis virus (VSV) only adapted to the novel hosts present during an evolution experiment (Turner and Elena, 2000). Host environment may be particularly important when the alternative phenotypes differ in niche width. Generalists benefit not just by being able to tap an uncontested resource: they also benefit by being able to access more of the available resources. When the generalist initially evolves and is rare, they benefit by having access to an uncontested

resource, enjoying low “intra-phenotypic” competition. However, as the generalists increase in frequency, competition among them increases for the novel host. At this point, generalists can no longer rely on the novel host for an advantage, and selection can act to increase the ability to utilize all available resources well. When search time is long, as in φ6, generalists benefit by decreasing their overall search time for a host cell, which can be achieved by increasing adsorption to the standard host.

Many theories, in particular ones that find coexistence between generalists and specialists and demonstrate sympatric speciation, assume that generalism comes with a cost—a jack-of-all-trades is a master of none (Levins, 1968; Lynch and Gabriel, 1987; Wilson and Yoshimura, 1994). Although host range expansion in φ6 has been associated with antagonistic pleiotropy, there was variation in antagonistic pleiotropy: the mean effect on standard host adsorption was weakly but significantly negative, but some host range expansion mutations were neutral or even improved adsorption to the standard host. Therefore, generalists evolved on the novel host alone would have experienced antagonistic pleiotropy on average, e.g. Ferris et al., (2006) and Duffy et al. (2006), but generalists evolved in a two-host environment would be under selection for the mutations that reduce or eliminate costs associated with search time: increases in adsorption rate to the standard host would decrease search time and increase fitness, as discussed in Chapter 3. When we removed the standard host and evolved the generalist on the novel host alone in allopatry, effectively removing selection against antagonistic pleiotropy, generalists decreased in adsorption to the standard host, and when it was maintained in sympatry, generalists increased in standard host adsorption.

Indeed, the difference in the host environment experienced by the generalist ancestor likely explains why novel host specialists evolved in Duffy et al. (2007) but not in our

experiment. Duffy et al. (2006) isolated a generalist ancestor from a random genetic screen on the novel host alone, which experienced antagonistic pleiotropy. In contrast, the generalist ancestor used in this experiment was *selected for* in a mixed host environment for at least 15 transfers (~45 generations, Bono et al., 2013) and consequentially was under strong selection to ameliorate antagonistic pleiotropy, as previously discussed in Bono et al. (2013) and Chapter 3. Since historical contingency can play a critical role in the subsequent evolution of key innovations (Blount et al., 2008, 2012), differences in choice of ancestor likely had a profound effect on the evolutionary outcome by altering the phenotypic space that the generalist could explore. It is possible that our generalist ancestor acquired mutations delaying or preventing novel host specialization in subsequent evolution even when evolved on the novel host alone for ~300 generations. Although we decreased the strength of competition from Bono et al. (2013) from MOI = 10^{-1} to 10^{-2}, which undoubtedly affected adaptation, we believe this selection to minimize antagonistic pleiotropy had a greater effect on the long term host range of generalists. Why? When Duffy et al. (2007) evolved novel host specialists from ancestors with antagonistic pleiotropy, the evolution of a novel host specialists was not contingent upon competition, and their host environment only contained the novel host, like our allopatric treatment, yet ours did not yield novel host specialists despite evolving for twice as long.

The appearance of the bull's-eye generalists was unexpected. Bull's-eye plaques have been reported in φ6 (Dessau et al., 2012) and other viruses, including lambda phage (Ohashi and Dove, 1976), P22 phage (Susskind, 1980) and respiratory syncytial virus (Numata et al., 2010). Bull's-eye plaque formation may reflect a delay between the initial phage contact to a cell and the final lysis of that cell, thereby increasing generation time and producing the distinctive fuzzy edges of the bull's-eye plaque (Abedon, 2008). As we were careful to avoid the bull's-eye

phenotype when selecting the generalist ancestor for this evolution experiment, we were surprised to observe them here. Interestingly, bull's-eye generalists were only observed in sympatry but never in allopatry, which we suspect is due to their high standard host specificity. Another possibility is that they arose from a new host range expansion mutation in a specialist. As for the stability of this three-way polymorphism, theory posits that the number of resource-use phenotypes cannot exceed the number of resources when resources are limited (Tilman, 1980, 1982), suggesting that one of the phenotypes will eventually be eliminated.

Future work should aim to resolve whether differences in generalist adaptation between sympatry and allopatry were due to access to the standard host and/or gene flow with specialists. Specifically, high throughput sequencing might help address the following issues: 1) identify the genetic basis of host range expansion and subsequent adaptation in sympatry and allopatry, 2) assess gene flow between the phenotypes, 3) determine if the bull's eye phenotype evolved from a generalist, specialist or hybrid of both, and 4) compare our generalists to the φ6 generalists that evolved to restrict their host range (Duffy et al., 2007).

Although generalists evolved differently in sympatry and allopatry, competition did not act as a wedge, driving generalists and specialists apart in host use, and novel host specialists were never detected. Despite the emphasis on gene flow as a disruptor of divergent selection, we found that a lack of antagonistic pleiotropy had a similar effect. Rather than competition acting as a wedge to separate alternative phenotypes in niche space, it selected for sympatric generalists to converge with the specialist in terms of higher adaptation to the standard host. We believe that this pattern is the result of differences in selection to minimize antagonistic pleiotropy in sympatry but not in allopatry. This study highlights the importance of the availability of resources and whether selection can act to minimize or ameliorate antagonistic pleiotropy, which

is often inherent in models of sympatric speciation (Dieckmann and Doebeli, 1999; Mallet, 2008; Nosil, 2008; Flaxman et al., 2013; Via, 2001). For instance, models often describe a scenario where the strength of competition declines with phenotypic distance, like beak size and seed type, e.g. (Dieckmann and Doebeli, 1999). This implicitly assumes that more phenotypically dissimilar individuals either cannot use the same resources or pay a cost for using them. Relaxing this likely has serious implications for branching events leading to sympatric speciation. Resource availability clearly has the potential to affect generalist evolution, and its selective potential to minimize or ameliorate antagonistic pleiotropy has distinct implications for theory requiring antagonistic pleiotropy as an integral part of adaptation to a novel resource.

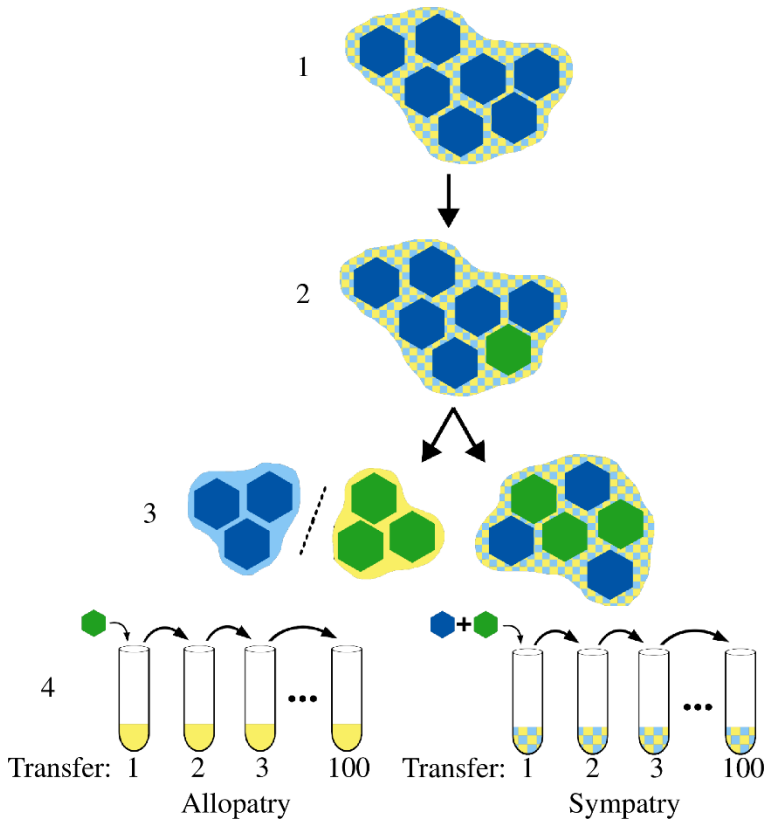

Figure 11. Experimental design.

We designed our experiments to parallel classic modes of geographic speciation. (1) Previously, the specialist (blue hexagon) ancestor was evolved on both the standard (light blue) and novel (yellow) hosts. (2) After several transfers, a generalist (green hexagon) evolved (Bono et al., 2013). (3) At the end of the previous evolution experiment, a generalist and specialist pair were isolated and used to found the sympatric and allopatric populations in this experiment. (4) Generalists were evolved for 100 transfers. In allopatry, the generalists were evolved alone on the novel host only. In sympatry, the generalists were evolved with a competitor (the specialist) on both hosts. Five replicate populations were evolved for each treatment.

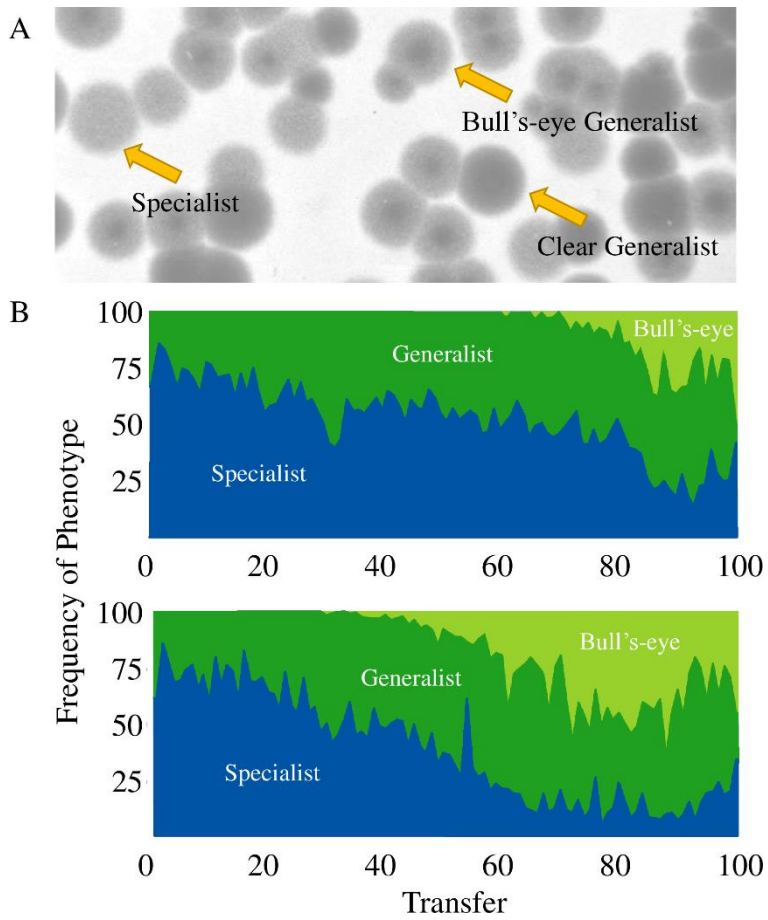

Figure 12. Evolution of the bull's-eye generalists.

(A) Photograph of the 3 phenotypes. (B) Frequencies of the phenotypes over time in the two sympatric populations in which the bullseye generalists evolve with specialists in blue, generalists (clear) in green and bullseye generalists in light green.

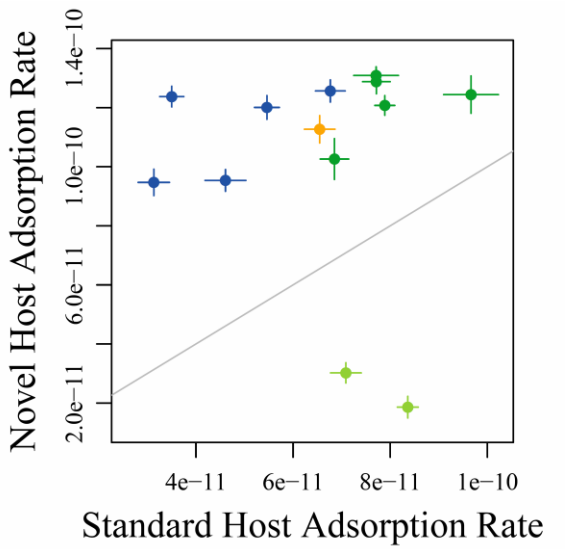

Figure 13. Adsorption rates of the evolved generalists.

Circles represent the mean ± SE of the adsorption rate of the generalists evolved in sympatry and allopatry from each replicate population. Specialists are shown in blue, clear generalists in green, bull's-eye generalists in light green, and ancestor in orange. Since there was a significant effect of the date on which the adsorption assay was performed, adsorption rates were corrected by subtracting the coefficient of the date effect

REFERENCES

Delbrück, M. and W. T. Bailey, Jr. 1946. Induced mutations in bacterial viruses. *Cold Spring Harbor Symposia on Quantitative Biology*, 11(1):33–37.

Abedon, S. T. 2008. *Bacteriophage ecology: population growth, evolution, and impact of bacterial viruses*, volume 15. Cambridge University Press.

Blount, Z. D., J. E. Barrick, C. J. Davidson, and R. E. Lenski. 2012. Genomic analysis of a key innovation in an experimental Escherichia coli population. *Nature*, 489(7417):513–518.

Blount, Z. D., C. Z. Borland, and R. E. Lenski. 2008. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 105(23):7899–7906.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster Nature*, 410(6827):463–466.

Bono, L. M., C. L. Gensel, D. W. Pfennig, and C. L. Burch. 2013. Competition and the origins of novelty: experimental evolution of niche-width expansion in a virus. *Biology Letters*, 9(1):20120616–20120616.

Chao, L. 1988. Evolution of sex in RNA viruses. *Journal of Theoretical Biology*, 133(1):99–112.

Chao, L. 1990. Fitness of RNA virus decreased by Mullers ratchet. *Nature*, 348(6300):454–455.

Chao, L. 1992. Evolution of sex in RNA viruses. *Trends in Ecology & Evolution*, 7(5):147–151.

Coyne, J. A., H. A. Orr, et al. 2004. *Speciation*, volume 37. Sinauer Associates Sunderland, MA.

Darwin, C. 1859. *On the origin of species by Charles Darwin: A facsimile of the first edition with an introduction by Ernst Mayr*. Harvard University Press.

Dessau, M., D. Goldhill, R. L. McBride, P. E. Turner, and Y. Modis. 2012. Selective pressure causes an RNA virus to trade reproductive fitness for increased structural and thermal stability of a viral enzyme. *PLoS Genetics*, 8(11):e1003102.

Dieckmann, U. and M. Doebeli. 1999. On the origin of species by sympatric speciation. *Nature*, 400(6742):354–357.

Duffy, S, P. E. Turner, and C. L. Burch. 2006. Pleiotropic costs of niche expansion in the RNA bacteriophage φ6. *Genetics*, 172(2):751–757.

Duffy, S., C. L. Burch, and P. E. Turner. 2007. Evolution of host specificity drives reproductive isolation among RNA viruses. *Evolution*, 61(11):2614–2622.

Ferris, M. T., P. Joyce, and C. L. Burch. 2006. High frequency of mutations that expand the host range of an RNA virus. *Genetics*, 176(2):1013–1022.

Flaxman, S. M., J. L. Feder, and P. Nosil. 2013. Genetic hitchhiking and the dynamic buildup of genomic divergence during speciation with gene flow. *Evolution*, 67(9):2577–2591.

Levins, R. 1968. *Evolution in changing environments: some theoretical explorations*, number 2. Princeton University Press.

Lynch, M. and W. Gabriel. 1987. Environmental tolerance. *The American Naturalist*, 129(2):283.

Mallet, J. 2008. Hybridization ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1506):2971–2986.

Malmberg, R. L. 1977. The evolution of epistasis and the advantage of recombination in populations of bacteriophage T4. *Genetics*, 86(3):607–621.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press.

Nosil, P. 2008. Speciation with gene flow could be common. *Molecular Ecology*, 17(9):2103–2106.

Numata, M., H. W. Chu, A. Dakhama, and D. R. Voelker. 2010. Pulmonary surfactant phosphatidylglycerol inhibits respiratory syncytial virus–induced inflammation and infection. *Proceedings of the National Academy of Sciences*, 107(1):320–325.

Ohashi, M. and W. F. Dove. 1976. Lethal lysogenization by coliphage lambda. *Virology*, 72(2):299–307.

Pfennig, D. W. and P. J. Murphy. 2003. A test of alternative hypotheses for character divergence between coexisting species. *Ecology*, 84(5):1288–1297.

Pfennig, D. W. and K. S. Pfennig. 2012. *Evolution's Wedge: Competition and the Origins of Diversity*, number 12. Univ of California Press.

Rosenzweig, M. L. 1978. Competitive speciation. *Biological Journal of the Linnean Society*, 10(3):275–289.

Rundle, H. D. and P. Nosil. 2005. Ecological speciation. *Ecology Letters*, 8(3):336–352.

Servedio, M. R., G. S. V. Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: 'magic' but not rare? *Trends in Ecology & Evolution*, 26(8):389–397.

Smith, T. B. and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.*, 27(1):111–133.

Susskind, M. M. 1980. A new gene of bacteriophage P22 which regulates synthesis of antirepressor. *Journal of Molecular Biology*, 138(4):685–713.

Tilman, D. 1980. Resources: A graphical-mechanistic approach to competition and Ppredation. *The American Naturalist*, 116(3):362.

Tilman, D. 1982. *Resource Competition and Community Structure.* Princeton University Press.

Turner, P. E. and L. Chao. 1999. *Nature*, 398(6726):441–443.

Turner, P. E. and S. F. Elena. 2000. Cost of host radiation in an RNA virus. *Genetics*, 156(4):1465–1470.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution*, 16(7):381–390.

Vidaver, A., R. Koski, and E. J. Van. 1973. Bacteriophage phi6: a lipid-containing virus of *Pseudomonas phaseolicola*. *J Virol*, 11:799–805.

West-Eberhard, M. J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press.

Wilson, D. S. and M. Turelli. 1986. Stable underdominance and the evolutionary invasion of empty niches. *The American Naturalist*, 127(6):835.

Wilson, D. S. and J. Yoshimura. 1994. On the coexistence of specialists and generalists. *The American Naturalist*, 144(4):692.

CHAPTER 5: CONCLUSIONS

The research I have performed for my thesis provides direct tests of competition as a key driver of both the origins and maintenance of diversity. Competition is widespread. Competition, particularly competition for resources, may promote divergence of character by selecting for individuals least like their competitors. In particular, individuals able to utilize a novel resource, even when toxic or inferior, have an advantage when competition for a preferred resource is strong. Over time, as these individuals continue to adapt to their novel resource, they may diverge phenotypically and genetically, becoming an alternative resource-use phenotypes. This could eventually lead to ecological speciation. Although many studies have found support for this route to diversification, relatively few studies have provided a direct experimental link between competition for resources and the evolution and maintenance of diversity.

Although field and experimental evidence has accumulated, relatively few studies have directly linked competition for resources with the origin and maintenance of diversity (but see Bolnick (2001) for a notable exception). In Chapter 2, I document competition as a key driver of the origins of novelty: populations experiencing strong competition for resources evolve the ability to utilize a novel resource faster and more often. Theoretical work in general and empirical studies specifically in φ6 (Duffy et al. 2006; Ferris 2007) suggest that "a jack-of-all-trades is a master of none", but we were unable to detect a cost to the generalists' host range expansion. Therefore, generalists tended to competitively exclude specialists in these populations, leading to no net change in diversity. Yet, in one population, generalists and

specialists coexisted under negative frequency-dependent selection, and generalist had a significantly higher specificity for the novel host, suggesting that niche partitioning was the responsible for coexistence. These results suggest that although competition can drive the origin of novel resource use, coexistence between generalist and specialist phenotypes is not guaranteed.

In Chapter 3, I address the central unanswered question from the previous chapter: can competition drive the maintenance as well as the origin of diversity? I repeated the evolution experiment as described in the previous chapter with one major exception: I reduced the ratio of the novel host. Using resource-ratio theory (Tilman 1980, 1982) as a guide, I altered the ratio of the hosts from 1:1 to 9:1 standard:novel hosts, simultaneously reducing the strength of competition for the standard host and the ecological opportunity for the novel host. This reduced selection for the generalists, slowing the evolutionary dynamics and allowing enough time for the specialist to evolve. By process of elimination, I determined that generalists paid a cost via antagonistic pleiotropy. Thus, the specialists were able to coexist through evolutionary rescue (Carlson et al. 2014). These results highlight the complexity of eco-evolutionary dynamics of coexistence in a relatively simple system, and we hope they inspire more rigorous investigation into the origins and maintenance of resource use polymorphisms in systems where antagonistic pleiotropy is not universal.

Theory suggests that after the evolution of a resource-use polymorphism, competition may continue to act as a wedge, driving phenotypes farther apart in niche space and potentially leading to speciation (West-Eberhard 2005; Pfennig and Pfennig 2012). In Chapter 4, I tested if the presence of a competitor can drive divergence in host use (sympatry) as compared to evolution on a novel resource alone (allopatry), mirroring the classic geographical modes of

speciation. We found that generalists evolved in sympatry and allopatry differed significantly in adsorption to the standard host but not the novel host. Most importantly, the sympatric generalists increased in attachment to the standard host rather than evolving to exclusive novel host use, like the allopatric generalists. With treatments differing in both access to the standard host and possible gene flow with the specialists, we cannot conclude which factor altered standard host adaptation. Future sequencing will help disentangle these confounding factors as well as the genetic and genomic basis of novel host use.

This body of work leaves several questions unanswered. First and foremost, sequencing is an obvious next step. As outlined at the end of Chapter 4, high throughput sequencing could help disentangle the confounding factors of gene flow and host environment. Additionally, this will uncover both the genetic and genomic basis of adaptation. Second, I would like to explore how our choice of ancestor affected the evolution, or lack thereof, of a novel host specialist. As previously discussed, the generalist ancestor used to found the sympatric and allopatric populations experienced selection to minimize antagonistic pleiotropy. If we selected a generalists with a different evolutionary history or one with fewer than generations on both hosts, would we have found similar results to Duffy et al. (2007)? On the other hand, does antagonistic pleiotropy reappear after prolonged absence of only novel host exposure? Finally, a big picture question that has long nagged me: what is the role, if any, of host range expansion in natural populations? These studies were confined to tightly controlled laboratory experiments. However, I have long wondered why φ6 can rapidly expand its host range. Is this simply the result of it being an RNA virus, or is it under selection to jump hosts in nature? One possible way to begin to assess this is to examine natural populations of φ6 and look for variation in host range. A few related studies suggest that lab studies are not unrelated to patterns observed in

natural populations. Lab-detected mutations linked to increased thermotolerance have been detected in natural populations (Paul Turner, personal communication), and since these mutations imposed antagonistic pleiotropy (Dessau et al. 2012), they are adaptive in these natural populations. This suggests that mutations arising in lab studies are not completely artificial but can also be selected for in natural populations. Also, variation in reassortment rate, one of the mechanisms hypothesized to be important for host range expansion, has been detected in natural populations (O'Keefe et al. 2010), opening the possibility that it is under selection.

Taken together, the studies in my dissertation support the hypothesis that competition can drive the evolution of a stable resource-use polymorphism. However, direct costs to expanded host range remained difficult or elusive to detect, despite previous studies documenting antagonistic pleiotropy in φ6. Rather, generalists evolved with both host experienced selection to minimize antagonistic pleiotropy, which has serious implications for theory that uses these costs as the basis for divergence. In conclusion, my research suggests that 1) competition alone can drive the origin of novel resource, 2) competition can lead to both the origin and maintenance of a resource-use polymorphism *even in the absence of antagonistic pleiotropy*, potentially by allowing sufficient time for the joint evolution of competitors; and 3) if variation in pleiotropy exists, selection can act to reduce or even ameliorate cost of niche width expansion. Taken together, these results show strong support for the hypothesis that competition drive the evolution of a stable resource-use polymorphism even in the absence of antagonistic pleiotropy.

REFERENCES

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410:463–6.

Carlson, S. M., C. J. Cunningham, and P. A. Westley. 2014. Evolutionary rescue in a changing world. Trends in Ecology & Evolution 29(9):521–530.

Dessau, M., D. Goldhill, R. L. McBride, P. E. Turner, and Y. Modis. 2012. Selective Pressure Causes an RNA Virus to Trade Reproductive Fitness for Increased Structural and Thermal Stability of a Viral Enzyme. PLoS Genetics 8(11):e1003102.

Duffy, S., C. L. Burch, and P. E. Turner. 2007. Evolution of host specificity drives reproductive isolation among RNA viruses. Evolution 61:2614–22.

Duffy, S., P. E. Turner, and C. L. Burch. 2006. Pleiotropic costs of niche expansion in the RNA bacteriophage phi 6. Genetics 172:751–7.

Ferris, M. T. 2007. Evolution of the host range of the bacteriophage φ6. University of North Carolina at Chapel Hill.

O'Keefe, K. J., O. K. Silander, H. McCreery, D. M. Weinreich, K. M. Wright, L. Chao, S. V. Edwards, S. K. Remold, and P. E. Turner. 2010. Geographic differences in sexual reassortment in RNA phage. Evolution 64:3010–3023.

Pfennig, D. W., and K. S. Pfennig. 2012. Development and evolution of character displacement. Ann. N. Y. Acad. Sci. 1256:89–107.

Tilman, D. 1982. Resource competition and community structure. Monogr. Popul. Biol. 17:1–296.

Tilman, D. 1980. Resources a graphical mechanistic approach to competition and predation. Am. Nat. 116:362–393.

West-Eberhard, M. J. 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. U. S. A. 102 Suppl :6543–6549.

APPENDIX A: SUPPLEMENTARY DATA FOR CHAPTER 2

Culture Conditions. Details of diluting, filtering, culture and storage of phage and bacteria appear in Ferris et al. (2006) and Duffy et al. (2005). All phage and bacteria were grown in LC medium (5 g/liter yeast extract, 10 g/liter bactotryptone, 5 g/liter NaCl) at 25°C. Prior to the start of each serial transfer or assay, hosts were grown to an OD_{600} corresponding to a density of $2x10^8$ cells/mL (*Ps phaseolicola*: $OD_{600} = 0.33$; *Pp ERA*: $OD_{600} = 0.12$; *Ps atrofaciens*, $OD_{600} = 0.15$; *Ps glycinea*, $OD_{600} = 0.3$).

We monitored the evolution of generalist phenotypes by plating phage population samples on lawns of 200 µL taken from a mixture of 1 mL of the standard host *Ps phaseolicola* and the following volume of the appropriate novel host: 10 µL *Pp ERA*, 50 µL *Ps atrofaciens* or 1 mL *Ps glycinea*. Generalist phage that gained the ability to use the novel host produced clear plaques, whereas the specialist ancestor produced turbid plaques.

Competition Assays. Generalist and specialist phage clones were isolated from populations of interest, mixed together at a particular ratio and incubated in conditions that exactly mimicked the evolution experiments described in the main text. The frequency of the generalist phage was determined by plating on mixed lawns at the beginning and end of each incubation and the fitness of the generalist relative to the specialist phage was calculated as $W = R_6/R_0$ where R_t is the ratio of generalists to specialists at time t hours.

Growth Rate Assays. Clonal phage isolates were mixed with a volume of exponentially growing *Ps phaseolicola* to achieve the same initial conditions (population sizes and densities) as used in the experiment in which the phage evolved, and incubated shaking at 25°C for 6 hours.

Intrinsic growth rate was calculated as $ln(P_6/ P_0)/6$, where P_t is the phage concentration at time t hours. To control for day effects, 5 replicate measures of the ancestor and of the evolved generalist and specialist phage from an individual lineage were obtained on a single day. Relative growth rates were then calculated by subtracting the ancestral mean from the specialist and generalist measures obtained on the same day.

Attachment Rate Assays. 2000 phage were mixed with 1 mL of approximately $2x10^8$ exponentially growing host cells and incubated shaking at 25°C. Initially, and after 40 minutes, 500 µL of this mixture was centrifuged at 6600 RPM to pellet the cells, and 200 µL of supernatant were plated to obtain a count of the free phage. The attachment rate constant was calculated as $k = -ln(P_{40}/P_0)/(40N)$ where N = number of host cells, and P_t is the number of free phage at time t, both determined by plating. We compared the attachment rate between the standard and novel hosts using a Student's paired t-Test.

Statistical comparison of evolution trajectories. We performed a parametric survival analysis using the *survreg* function in R v. 2.13.0 to examine the effects of competition treatment, population size, and novel host identity on the time at which generalists first arose within the population. We also examined models that compared the day on which generalist comprised 5%, 10%, 20% or 50% of the population, but found that it did not qualitatively affect the analysis results. We right censored lineages in which generalists did not achieve the specified frequency, which is a way of accounting for time until an event (the detection of generalists) in lineages in which the event did not occur, used an exponential distribution for the hazard function (i.e.

assumed that mutations arose at a constant rate), and used the Akaike Information Criterion (AIC) to choose the model that best fit the data.

Table 1. Analysis of deviance from a survival analysis of the 18 lineages.

Data shown in Figure 2.

	df	Deviance (χ^2)	Residual df	-2*LL	*p*-value
NULL	NA	NA	17	85.91901	NA
Competition	1	5.8515864	16	80.06743	0.0155630159
Population size	2	0.4765206	14	79.59091	0.7879975619
Novel Host	2	21.6022568	12	57.98865	0.0000203765
Competition *Population Size	2	0.0712390	10	57.91741	0.9650074067
Competition *Novel Host	2	5.3000698	8	52.61734	0.0706487475
Population Size*Novel Host	4	2.0977468	4	50.51959	0.7177863425

Table 2. Analsysis of deviance table from a survival analysis of the 30 lineages.

Data shown in Figure 3.

	df	Deviance (χ^2)	Residual df	-2*LL	*p*-value
NULL	NA	NA	32	191.6709	NA
Population Size	2	1.574375	30	190.0965	0.455123
Novel Host	2	9.182161	28	180.9143	0.010142
Population Size*Novel Host	4	2.123052	24	178.7913	0.713139

Table 3. Linear mixed effects model model of growth rate.

Data in Figure 17, with novel host and phenotype (generalist or specialist) modeled as fixed effects, and lineage modeled as a random effect.

	Num df	**Den df**	***F***	***p*-value**
(Intercept)	1	250	3931.163	<.0001
Novel Host	1	25	0.677	0.4185
Phenotype	1	250	8.592	0.0037
Novel Host*Phenotype	1	250	0.351	0.554

Table 4. Attachment rates of evolved generalists.

Novel host	N^a	$k_{novel}{}^b$	$k_{standard}{}^b$	p-value[c]	$Pr(novel)^d$
Pp ERA	10^5	$7.8\times10^{-12} \pm 1.6\times10^{-11}$	$1.0\times10^{-10} \pm 2.7\times10^{-11}$	3.82×10^{-5}	7.2%*
Pp ERA	10^6	$-5.2\times10^{-13} \pm 3.7\times10^{-11}$	$1.7\times10^{-11} \pm 3.5\times10^{-11}$	0.413	0%
Pp ERA	10^7	$1.3\times10^{-10} \pm 4.6\times10^{-11}$	$5.3\times10^{-11} \pm 3.5\times10^{-11}$	9.42×10^{-4}	72%*
Ps atrofaciens	10^5	$-9.6\times10^{-13} \pm 8.4\times10^{-12}$	$8.4\times10^{-11} \pm 2.3\times10^{-11}$	6.72×10^{-4}	0%*
Ps atrofaciens	10^6	$4.6\times10^{-13} \pm 1.9\times10^{-11}$	$1.0\times10^{-10} \pm 3.0\times10^{-11}$	0.00161	0.46%*
Ps atrofaciens	10^7	$1.2\times10^{-11} \pm 1.3\times10^{-11}$	$1.0\times10^{-10} \pm 4.1\times10^{-11}$	2.14×10^{-4}	11%*
Ps glycinea	10^5	$8.4\times10^{-11} \pm 2.0\times10^{-11}$	$1.2\times10^{-10} \pm 2.0\times10^{-11}$	0.0144	40%*
Ps glycinea	10^6	$2.0\times10^{-11} \pm 9.1\times10^{-12}$	$1.1\times10^{-10} \pm 2.0\times10^{-11}$	4.45×10^{-4}	16%*
Ps glycinea	10^7	$1.0\times10^{-11} \pm 1.0\times10^{-11}$	$1.2\times10^{-10} \pm 3.2\times10^{-11}$	0.232	46%

[a] Transfer population size.

[b] Measures are given in mean ± standard deviation.

[c] Statistical probability that k_{novel} is not different from $k_{standard}$.

[d] The proportion of phage that attach to (i.e. infect) the novel host was calculated as $Pr(novel) = k_{novel} / (k_{novel} + k_{standard})$ by assuming that the novel and standard hosts are present at equal densities.

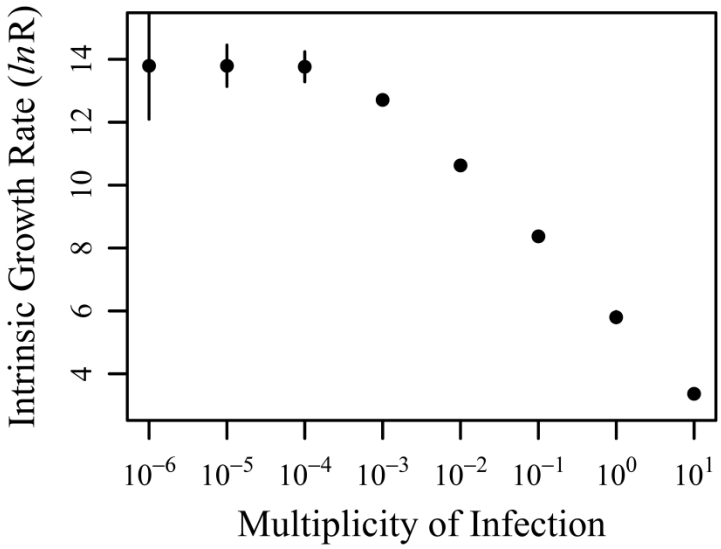

Figure 14. Effect of competition on intrinsic growth rate.

We manipulated the strength of intraspecific competition by increasing the multiplicity of infection (MOI). Phage were incubated with the standard host *Ps phaseolicola* at each MOI for 6 hours. Data are means and 95% confidence intervals based on three replicate measures at each MOI. The decrease in intrinsic growth rate as MOI increases confirms that higher MOIs impose stronger intraspecific competition. Note that the intrinsic growth rate in our strong competition treatment (MOI = 10^{-1}) is significantly lower than in our weak competition treatment (MOI = 10^{-3}).

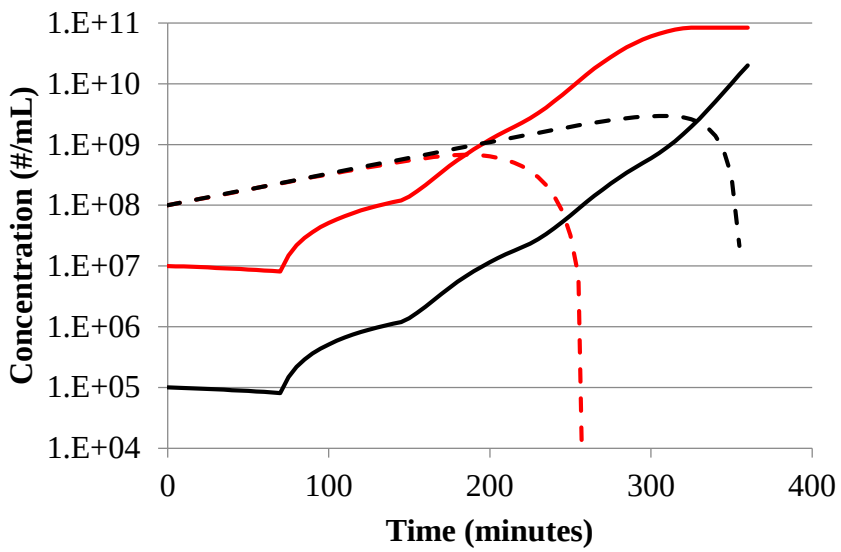

Figure 15. Predicted growth dynamics under strong and weak competition.

Red lines show predictions under strong (red, initial MOI = 10^{-1}), and black lines show predictions under weak competition (black, initial MOI = 10^{-3}). Lines show the estimated concentration of the wild type ϕ6 phage (solid) and of the standard laboratory host *Ps phaseolicola* (dashed) over the course of a 6 hour incubation. Dynamics were estimated in the manner of (Abedon et al., 2001, *Applied Environmental Microbiology*, 67: 4233-4241) based on our measurements of ϕ6 attachment rate ($k = 2\text{x}10^{-11}$), lysis time (L = 70 minutes), and burst size (B = 70 phage). Although we did not collect empirical data for intermediate timepoints, the estimated phage concentrations at 6 hours are a close match to our empirical observations in Figure S1. In both cases, the MOI increases over the course of 6 hours, reaching an MOI = 1 when the solid and dashed lines cross. In the strong competition treatment, MOI>1 for the last half of the incubation. In the weak competition treatment, MOI>1 only for a short period at the end of the experiment. Phage that infect hosts during this time do not have enough time to produce progeny before the incubation is halted and hosts (whether infected or not) are removed from the culture.

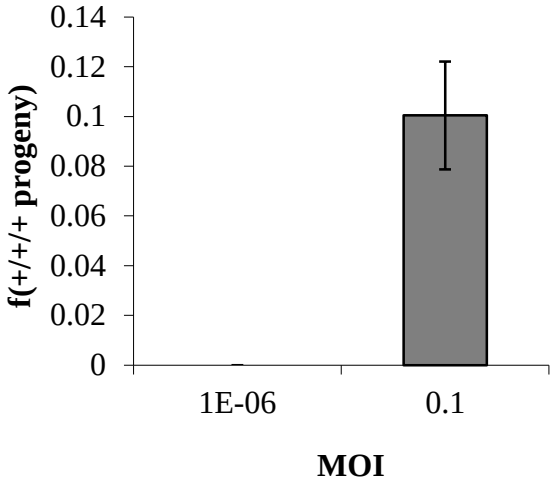

Figure 16. The rate of amphimixy increases with MOI on the standard host.

The ϕ6 genome consists of three linear segments. Although homologous recombination does not occur, reassortment of the segments when two phage co-infect a single host results in amphimixy. We assessed the effect of increasing MOI on the rate of reassortment using a 1:1 mixture of phage with a β-galactosidase marker on the large genome segment (denoted X/+/+) and phage with the same marker on the medium genome segment (+/X/+). We incubated this mixture with the standard host *Ps phaseolicola* for 6 hours at either low or high MOI, and then counted the reassortant unmarked (+/+/+) progeny by plating. Reassortant progeny are rare at low MOI, but occurred at a frequency of 10% at MOI = 10^{-1} (our strong competition treatment). *Note, however, that reassortment is expected to affect the rate of adaptation only after genetic variation has accumulated. Our main result was that competition affected the time at which the first generalist mutant was observed. Before this time, generalist mutations were too rare to be affected by reassortment.*

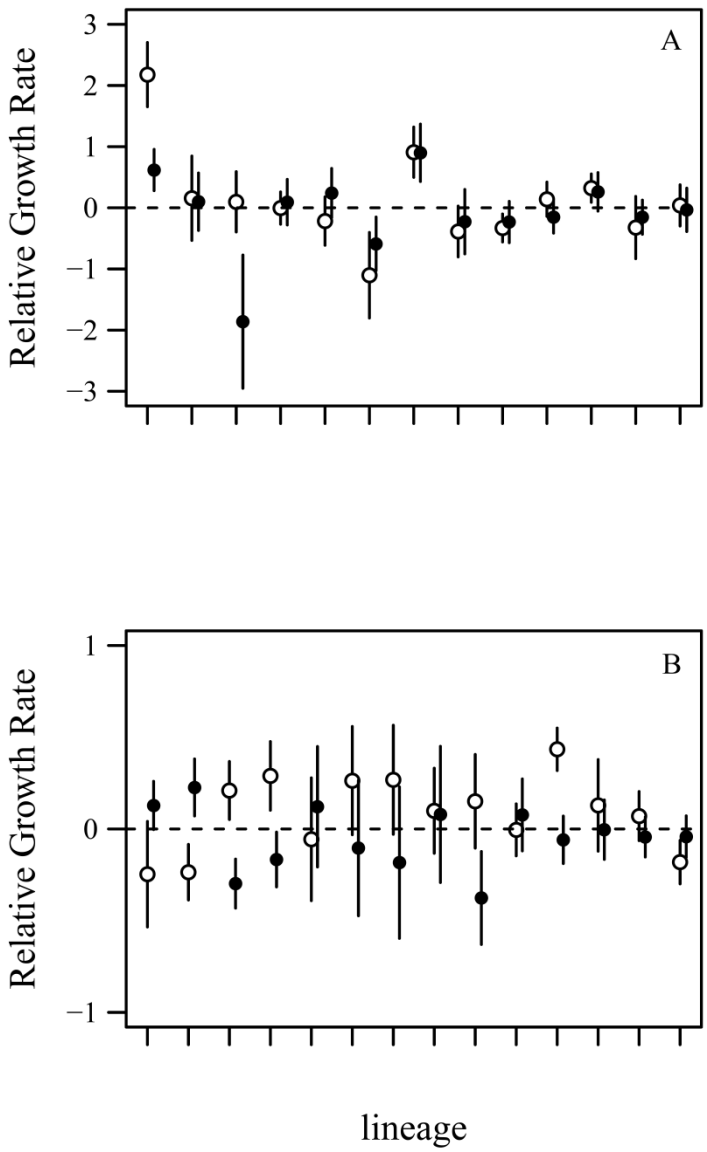

Figure 17. Costs of generalism.

Data are relative growth rate means and standard errors based on 5 replicate measures on the standard host *Ps phaseolicola* in the strong competition conditions in which these phage evolved (Figure 1). We show the growth rates relative to the ancestor of evolved generalists (closed circles) and specialists (open circles) for all lineages in which a generalist evolved on the novel hosts (A) *Ps atrofaciens* and (B) *Pp ERA*. We measured only lineages where generalists arose but did not achieve fixation.