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Evolutionary biology

Competition and the origins of novelty: experimental evolution of niche-width expansion in a virus

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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 use a wider range of resources, possibly even using entirely novel resources that are less in demand. Yet, there have been few experimental tests of these ideas. Using the bacterial virus (bacteriophage) $\phi 6$ as a model system, we examined whether competition for host resources promotes the evolution of novel resource use. In the laboratory, $\phi 6$ exhibits a narrow host range but readily produces mutants capable of infecting novel bacterial hosts. Here, we show that when $\phi 6$ populations 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.

1. 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 [1,2]. Specifically, theory holds that in a population experiencing intense competition, frequency-dependent selection will favour individuals that can exploit an underused resource—even if this resource is novel and of lower quality—because these individuals will experience decreased competition [3,4]. Such selection has long been viewed as crucial in promoting niche-width expansion [5], resource polymorphism [6] and even speciation and adaptive radiation [2,7]. Yet, much of the empirical support for this theory is indirect [2,7]. Indeed, relatively few experimental studies have established a causal link between resource competition and niche-width expansion [5].

Here, we present such an experimental test. Using a rapidly evolving bacteriophage, $\phi 6$ (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: (i) that rare resource-use phenotypes would be favoured over common ones, yielding frequency-dependent selection [8]; (ii) that generalists using 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 [5]; and (iii) that novel resource use would evolve faster in larger populations, owing to a greater supply of adaptive mutations [9]. 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.

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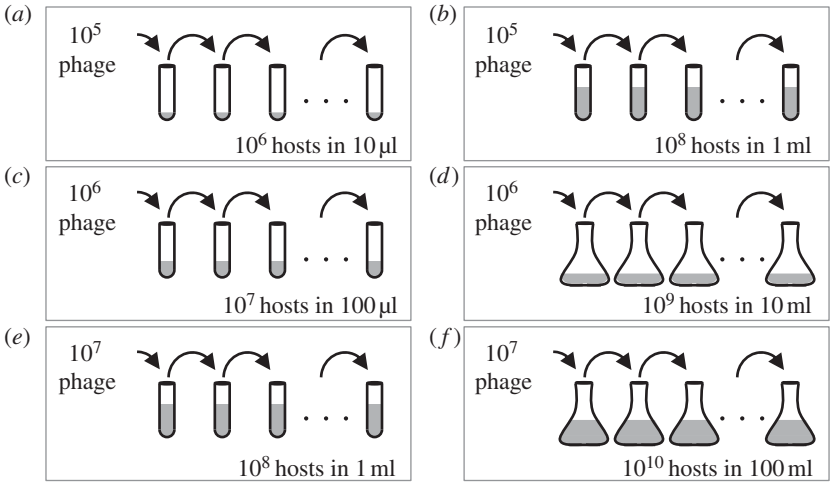

Figure 1. Experimental design. (*a*,*c*,*e*) Competition: inital MOI = 10^{-1}; (*b*,*d*,*f*) no competition: initial MOI = 10^{-3}.

2. 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^{-1} (figure 1). Individual lineages were subjected to either strong (figure 1*a*,*c*,*e*) or weak (figure 1*b*,*d*,*f*) intraspecific competition by initiating each transfer at a ratio of phage to hosts (multiplicity of infection, MOI), of 10^{-3} or 10^{-1}, respectively. This increase in MOI reduces the intrinsic growth rate of $\phi 6$ ($\ln(P_6/P_0)$; see §3) from 12.7 to 8.4 per transfer (see the electronic supplementary material, figure S1; additional effects of high MOI are discussed in the electronic supplementary material, figures S2 and S3). 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 1*a*) and 100 ml (figure 1*f*) to achieve a constant host density across treatments despite differences in MOI and phage population size. Cultures were incubated with shaking at 25°C for 6 h 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 three novel hosts—*Pseudomonas syringae* pathovar *glycinea*, *P. syringae* pathovar *atrofaciens* or *P. pseudoalcaligenes* pathovar ERA. These hosts were chosen because $\phi 6$ requires only a single point mutation to infect them [10,11]. In laboratory culture, their growth rates differ (doublings per hour = 0.19 on *Ps. glycinea*, 0.26 on *Ps. atrofaciens* and 0.45 on *Pp. ERA*, compared with 0.35 on the standard host *Ps. phaseolicola*), and generalist mutant phage exhibit different growth rates on three hosts. The median ($\pm$ s.d.) intrinsic growth rate of three independent generalist mutants ranged from 7.80 $\pm$ 0.59 on *Ps. glycinea* to 5.78 $\pm$ 4.46 on *Pp. ERA* to 3.93 $\pm$ 1.27 on *Ps. atrofaciens*. Thus, the novel hosts differ in quality with *Ps. glycinea* > *Pp. ERA* > *Ps. atrofaciens*.

3. Material and methods

(a) Strains and culture conditions

The RNA bacteriophage $\phi 6$ used in this study is a laboratory strain descended from the original isolate [12]. The bacterium *Pseudomonas syringae* pathovar *phaseolicola* strain HB10Y [13] 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 [13]. 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 the electronic supplementary material).

(c) Attachment rate assays

We measured the rate at which phage bind to the standard and novel hosts (see the electronic supplementary material).

(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 the electronic supplementary material).

(e) Data archive

All data are archived at datadryad.org.

4. Results

As predicted, when $\phi 6$ 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 ($\chi^2 = 21.60$, d.f. = 2, $p < 0.0002$; full analysis in the electronic supplementary material, table S1) 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

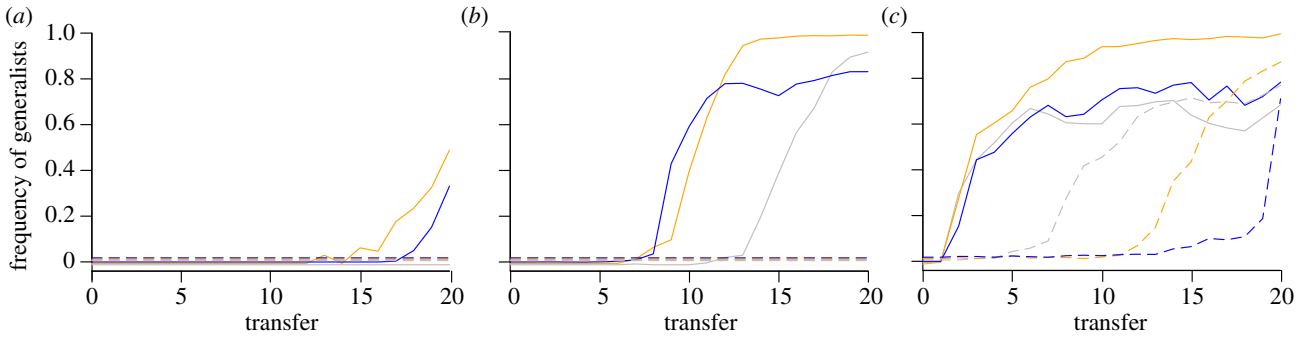

Figure 2. Effects of competition and novel host treatments on host range expansion. Lines show the frequency of generalists over time in lineages evolved under weak (dashed lines) or strong (solid lines) competition, transfer population sizes of $N = 10^5$ (grey), 10^6 (orange) or 10^7 (blue), and novel host (*a*) *Ps. atrofaciens*, (*b*) *Pp. ERA* or (*c*) *Ps. glycinea*. Lines are offset vertically to be distinguishable.

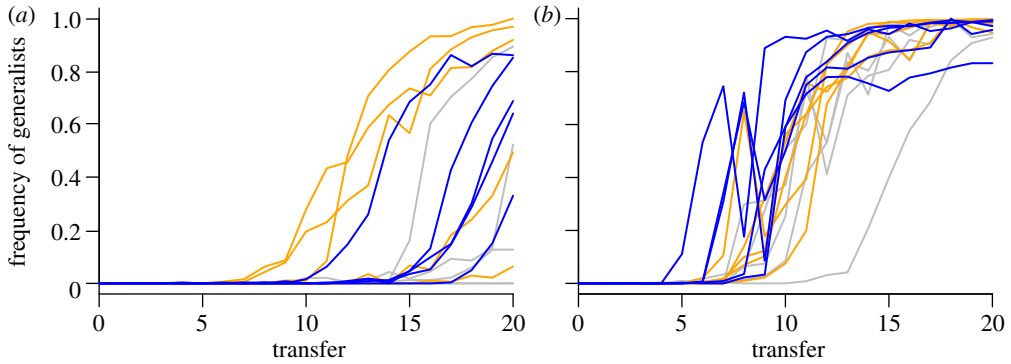

Figure 3. Effect of population size on host range expansion in microcosms containing novel hosts (*a*) *Ps. atrofaciens* and (*b*) *Pp. ERA*. Lines show the frequency of generalists over time in lineages evolved at transfer population sizes of $N = 10^5$ (gray), 10^6 (orange) or 10^7 (blue).

($\chi^2 = 5.85$, d.f. $= 1$, $p = 0.0156$). 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 (see figure 3 and electronic supplementary material, table S2; $\chi^2 = 1.57$, d.f. $= 2$, $p = 0.4551$).

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 $t_{121.208} = 7.457$, $p = 7.31 \times 10^{-12}$; figure 4). However, the high fitness of generalists when common ($\ln(W) \geq 0$ 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 ($p = 0.0037$, $F_{1,250} = 8.592$; electronic supplementary material, table S3), but this overall difference was owing to a large fitness cost paid by generalists in only a few lineages (2/13 on *Ps. atrofaciens* and 4/14 on *Pp. ERA*, electronic supplementary material, figure S4).

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. On the basis of 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 $Pr = k_{\text{novel}}/(k_{\text{standard}} + k_{\text{novel}}) = 0.26$ for *Pp. ERA*, 0.04 for *Ps. atrofaciens*, and 0.34 for *Ps. glycinea*, see the electronic supplementary material, table S4). Only one *Pp. ERA* lineage was significantly more likely to infect the novel host ($Pr = 0.72$; $p = 0.0009$).

5. Discussion

Intraspecific competition for resources has long been regarded as a key agent of divergent selection [2]. In some cases, competition may even promote the evolution of novel resource-use phenotypes, although few experiments

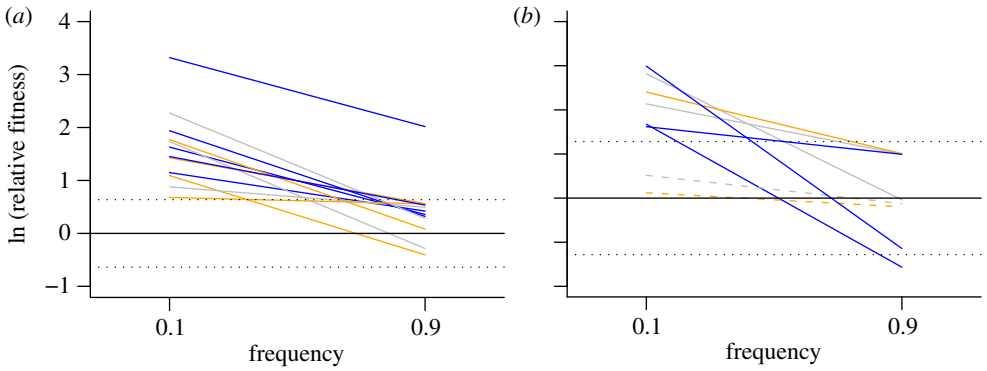

Figure 4. Frequency-dependent selection. Lines correspond to independent lineages evolved in microcosms containing (*a*) *Ps. atrofaciens* and (*b*) *Pp. ERA* in which the specialists were still present after 20 transfers. We show the fitness of generalists relative to specialists measured in competition assays initiated at generalist frequencies of 0.1 and 0.9. Line colour and style corresponds to the representation in figure 2. Dotted horizontal lines are the 95% CI surrounding zero, calculated using the experiment-wide mean squared error (i.e. Fisher's least significant difference).

have demonstrated this [5]. We sought to fill this gap by using a rapidly evolving bacteriophage, $\phi 6$. 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 $\phi 6$ 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 the population size (figure 3), indicating that genetic variation (i.e. mutation supply) was not limiting 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 competition-mediated selection [14]. Although fitness was indeed frequency-dependent (figure 4), 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 [15]. Here, temporal variability was manifest as a change in the probability that a generalist phage infects a novel host over the time course 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 uses an untapped resource can persist—even when it is poorly adapted to that novel resource—if competition for the preferred resource is strong.

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