Coevolution's conflicting role in the establishment of beneficial associations

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Reciprocal adaptation between hosts and symbionts can drive the maintenance of symbioses, resulting in coevolution and beneficial genotypic interactions. Consequently, hosts may experience decreased fitness when paired with nonsympatric partners compared to sympatric symbionts. However, coevolution does not preclude conflict—host and symbiont can act to advance their own fitness interests, which do not necessarily align with those of their partner. Despite coevolution's importance in extant symbioses, we know little about its role in shaping the origin of symbioses. Here, we tested the role of coevolution in establishing a novel association by experimentally (co)evolving a host with a protective bacterium under environmental stress. Although evolution in the presence of nonevolving bacteria facilitated host adaptation, co-passaged hosts did not exhibit greater adaptation rates than hosts paired with nonevolving bacteria. Furthermore, co-passaged hosts exhibited greater fecundity when paired with sympatric, co-passaged bacteria compared to co-passaged bacteria with which they did not share an evolutionary history. Thus, shared evolutionary history between the hosts and microbes actually reduced host fitness and has the potential to impede evolution of new beneficial associations.

The success of many long-term beneficial symbioses has been attributed to coevolution between host and symbiont. Reciprocal selection between partners can affect both species at the molecular level, leading to complementarity of genomes and symbiosis-dependent variation in gene expression (Toft and Andersson 2010; Suen et al. 2011; Heath et al. 2012; Wilson and Duncan 2015). The resulting divergence in evolutionary trajectories across populations creates specificity between the host and its symbiont (Murfin et al. 2015; Parker et al. 2017). Drawing from past studies on extant symbioses, we might expect that coevolution plays an important role in the evolution of novel beneficial associations by helping hosts obtain optimum fitness gains from associating with particular microbes.

Although extant model systems have provided evidence for the results of long-term coevolutionary interactions, it is difficult to directly test for the role of coevolution in shaping host and symbiont evolutionary trajectories (Janzen 1980; Moran and Sloan 2015). Experimental (co)evolution experiments have provided an effective solution to this dilemma (Hoang et al. 2016).

These experiments have illuminated the critical role of coevolution in the formation and evolutionary paths of partners in association—by reducing antagonism in parasites and increasing benefits between mutualists, co-passaging can promote rapid adaptation to stressful environments (Hillesland and Stahl 2010; Gibson et al. 2015; Rafaluk-Mohr et al. 2018). Nonetheless, repeated interactions between partners do not necessarily prevent exploitation, where the more rapidly evolving microbe is predicted to gain an advantage over the slower evolving host (Sachs et al. 2011). However, there have been few empirical studies on the origin of symbiosis in general (Hoang et al. 2016). Experimental evolution approaches are thus poised to address whether reciprocal selection between host and microbe can facilitate or hinder the evolution of novel beneficial associations.

Establishing associations with novel microbes can confer hosts with additional traits, allowing hosts to adapt to environments to which they otherwise cannot do by themselves. Previous work has found that *Caenorhabditis elegans* nematodes passaged in the presence of nonevolving, protective *Bacillus subtilis* bacte-
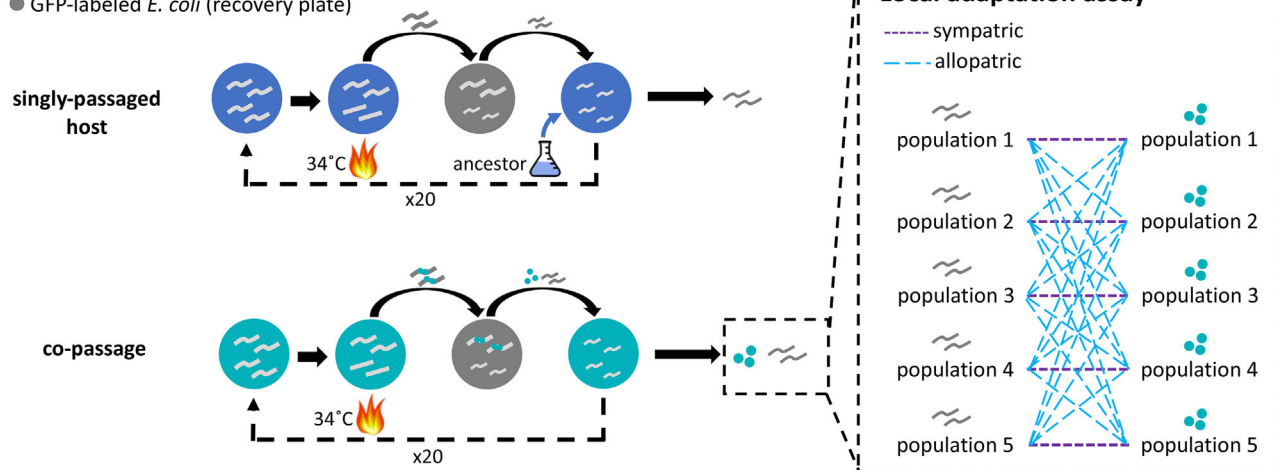
Figure 1. Setup of experimental evolution. Hosts were passaged in the presence of ancestral *B. subtilis* (singly passaged treatment) or co-passaged with *B. subtilis* (co-passaged treatment) for 20 generations, with five replicate populations each. Each treatment contained a recovery period after heat stress where nematodes produced progeny on GFP-labeled *E. coli*. Local adaptation assays consisted of combinations of sympatric hosts and bacteria that shared an evolutionary history during experimental evolution, and allopatric combinations that did not share an evolutionary history.

ria under heat stress evolved the greatest fitness compared to nematodes in the absence of the bacteria or the heat stress (Hoang et al. 2021). In the current study, we asked how co-passaging, which allows for coevolution, affects the evolutionary trajectory of a nascent beneficial association. We co-passaged *C. elegans* and *B. subtilis* under heat stress for 20 generations of selection, each treatment having five replicate populations (Fig. 1). We first compared fitness of hosts that were passaged with nonevolving bacteria (singly passaged hosts) with hosts that were co-passaged with bacteria (co-passaged hosts). We then tested the specificity of the interaction by pairing co-passaged hosts either with their sympatric, co-passaged bacteria or co-passaged bacteria that did not share their evolutionary history. Overall, we find that co-passaging resulted in host–bacteria specificity, where sympatric combinations produced fewer nematode offspring, indicating that sympatric bacteria hinder host adaptation to stress.

Methods

STRAINS AND MEDIA

Bacillus subtilis strain 168, generously provided by I. Gusarov, was used as the starting bacterial strain for experimental evolution (Gusarov et al. 2013). All evolved bacterial populations came from one ancestral *B. subtilis* 168 colony. Nematode strain N2 Bristol and *E. coli* strains OP50 and OP50-GFP were provided by the Caenorhabditis Genetics Center, which is funded by the NIH

Office of Research Infrastructure Programs (P40 OD010440). The ancestral *Caenorhabditis elegans* host was composed of roughly 93.7% hermaphrodites and 6.3% males. To generate this population (LTM-EE1), we independently mutated four populations of *C. elegans* N2 using ethyl methane-sulfonate (catalog #M0880, Sigma–Aldrich, St. Louis, MO) following Morran et al. (2011), then combined and froze the four populations to establish a single genetically variable ancestral host population to facilitate a response to selection. For all experiments, we grew *B. subtilis* on Nematode Growth Medium Lite (US Biological, Swampscott, MA) containing 2% glucose and 0.5 mM arginine (NGMga). We grew GFP-labeled OP50 (OP50-GFP) on NGM Lite.

Method details
Experimental evolution: Co-passage treatment
We co-passaged hosts and bacteria together under heat stress (five replicate populations). We surface sterilized the ancestral host eggs using an established alkaline hypochlorite protocol (Stiernagle 2006) and put roughly 500–700 larvae onto *B. subtilis*, kept at 20°C. Once the nematodes reached adulthood (after 3 days), we heat shocked nematodes at 34°C for 6 h. The plates were then left on the benchtop to cool down for 20 min, after which all nematodes were washed with M9 and transferred to OP50-GFP, where they were kept at 20°C until the following day. We picked a maximum of 40 live nematodes to crush (there were not 40 nematodes that survived to the next day on occasion) and plated

them onto LB containing 10 µg/mL streptomycin to limit OP50-GFP growth. We left the remaining hosts to produce offspring on OP50-GFP plates at 20°C. We used OP50-GFP as the recovery bacterium due to its phenotypic difference from *E. coli* OP50 and *B. subtilis* 168, while still being neutral with respect to its effects on *C. elegans* under standard conditions. After the *B. subtilis* extracted from heat shocked hosts were incubated at 28°C, we inoculated at most 20 colonies into LB broth, then plated 100 µL of the overnight culture onto NGMga. We then washed nematodes off the OP50-GFP plates and aliquoted roughly 500–700 larvae onto the *B. subtilis* lawn. We then heat shocked them 3 days later to start the next passage. After 10 passages, we froze each population, then revived them again to resume the experiment. After 20 passages, we froze each population, then thawed them to conduct host fitness assays. We designate this the "co-passaged" treatment.

Experimental evolution: Singly passaged host treatment

Concurrently, we passaged hosts in the presence of the ancestral *B. subtilis* under heat stress (five replicate populations). After heat shock, we let nematodes produce offspring on OP50-GFP and aliquoted roughly 500–700 larvae onto ancestral *B. subtilis* to start the next passage. We designate this the "singly passaged" treatment, which is from the same stock of B+H+ hosts of the previous study (Hoang et al. 2021).

Assay: Host survival and fecundity

Following the protocols described in Hoang et al. (2021), we conducted assays to evaluate host survival and fecundity as measures of fitness. For comparisons between co-passaged hosts and singly passaged hosts on co-passaged and ancestral bacteria, we paired co-passaged hosts with either the bacterial population with which they were co-passaged (i.e., sympatric bacteria) or the ancestral bacteria, and each singly passaged host population with one of the co-passaged bacteria or the ancestral bacteria (2 hosts × 2 bacteria × 5 experimental evolution replicate populations × 3 replicate plates). We followed the schedule for one passage of experimental evolution, as described above, for each of the evolved replicate populations, heat shocking about 100–200 nematodes. After heat shock, we quantified the number of live hosts by prodding nematodes with a platinum pick to determine signs of movement, then transferred the nematodes to OP50-GFP and maintained them at 20°C. We quantified the number of offspring produced per heat shocked adult 2 days later. For each replicate population, we heat shocked three replicate plates, totaling 48 plates (3 evolved bacteria × 5 experimental evolution replicate populations × 3 replicate plates) + (1 ancestral bacteria × 3 replicate plates).

To assess local adaptation, we paired each co-passaged host population with either their sympatric bacteria or with the other four bacterial populations (Fig. 1). To assess host fitness, we followed the schedule for one passage of experimental evolution and quantified survival and fecundity as described above. We conducted two independent sets of experiments: one set for the overall comparison between sympatric (5 combinations), allopatric (20 combinations), and ancestral pairings, and one set for the fine-scale population-level comparisons. For each combination, we heat shocked three replicate plates, totaling 78 plates for the first set of experiments and 75 plates for the second set. All host fitness assays were conducted for three rounds, with each round including all populations from all treatments. To determine bacterial fitness within nematodes, we crushed individual nematodes following a previously established protocol to determine *B. subtilis* abundance in vivo (Vega and Gore 2017). Briefly, we washed nematodes off their heat shocked plates with M9 into Eppendorf tubes, then washed them three times with cold 0.01% Triton-X 100 in M9. After incubation at 4°C for 15 min, we soaked them in 1:1000 diluted bleach for 15 min at 4°C to further remove surface bacteria. We then soaked nematodes in 0.25% sodium dodecyl sulfate (SDS) + 3% dithiothreitol (DTT) for 20 min, then transferred nematodes to a 96-well plate containing sterile silicon carbide grit and 0.01% Triton X-100 in M9. We then disrupted the samples using a Qiagen TissueLyser II homogenizer, plated out the samples onto LB plates, and quantified the number of colony-forming units (CFUs) 2 days later. We heat shocked one plate for each sympatric and allopatric combination, crushing six to eight individuals from each plate. In addition to these pairings, we also quantified ancestral bacterial growth in co-passaged hosts to control for host environment. In total, we quantified bacterial growth for about 180–240 nematodes (5 sympatric combinations + 20 allopatric combinations + 5 co-passaged hosts, 6–8 individuals each). We conducted the bacterial fitness assay for three rounds.

STATISTICAL ANALYSIS

To compare survival and fecundity across co-passaged and singly passaged hosts, we conducted a linear mixed model for each fitness measurement. We analyzed the total fecundity means by taking the mean of each replicate population from each experimental evolution treatment, determined by averaging the technical replicates for a given population within a given round. The main effects of bacteria (co-passaged or ancestral), host (co-passaged or singly passaged), and their interaction were treated as fixed terms. Round and replicate population (nested within host) were treated as random effects. To analyze the overall level of local adaptation, we tested the main effect of pairing (sympatric, allopatric, or ancestral) on host survival and fecundity using linear mixed models, and similarly for bacterial abundance. Round and pairing × round interaction were treated as random effects. We then performed Student's *t*-tests (pairwise post hoc tests within the analysis) to compare means between treatments.
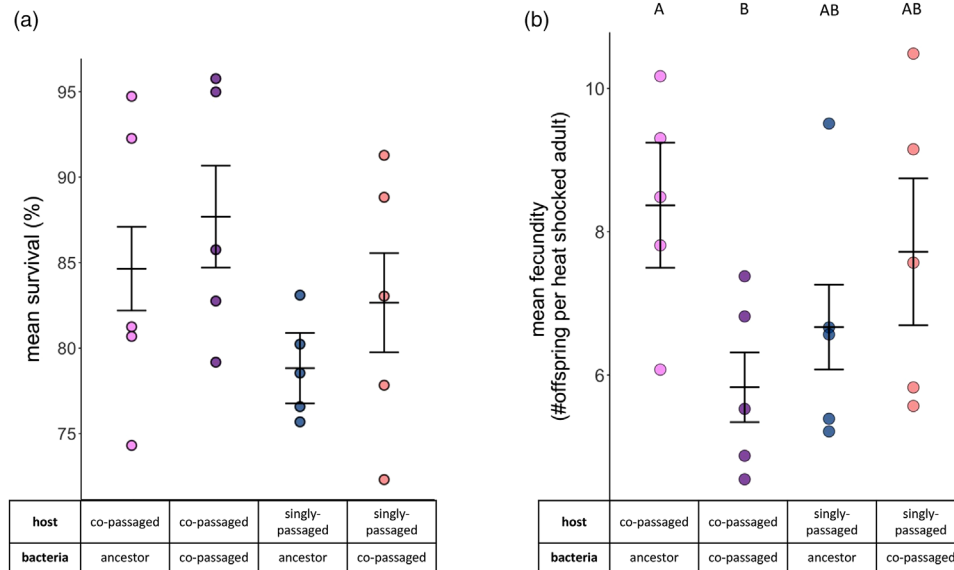
Figure 2. Fitness of singly passaged and co-passaged hosts. Evolved hosts were heat shocked at 34°C for 6 h on the indicated bacteria, after which (a) survival and (b) fecundity were measured. The *x*-axes indicate the host–bacteria combination that underwent heat shock after experimental evolution. Each plate contained about 100–200 nematodes. Each data point represents the mean for one replicate population from experimental evolution. The data are combined across three rounds. Error bars indicate standard errors. Treatments that are not the same letter are significantly different.

For fine-scale local adaptation assays, we used a generalized linear model (GLM) with Poisson distribution and log link function using maximum likelihood estimation. We tested the effects of bacterial population, host population, their interaction, round, round × bacterial population interaction, and round by host population interaction. We then performed linear contrast tests (mechanisms within the analysis for comparing variables within an effect in the model) to compare each sympatric combination against all possible combinations of allopatric pairings involving the focal host and bacterial populations (Morran et al. 2014; Gibson et al. 2015). All analyses were conducted using JMP Pro 14.

Results

CO-PASSAGING DOES NOT FACILITATE HOST ADAPTATION TO STRESS

Past studies have shown that partners can evolve toward greater benefits when co-passaged with one another (Hillesland and Stahl 2010; Rafaluk-Mohr et al. 2018). Consequently, we predicted that hosts and bacteria would evolve beneficial interactions, providing greater fitness gains for co-passaged hosts compared to singly passaged hosts. We found no overall significant difference in terms of host survival (host: $F_{(1,8)} = 1.99$, $P = 0.2$; bacteria: $F_{(1,28)} = 3.23$, $P = 0.08$; interaction: $F_{(1,28)} = 0.04$, $P = 0.84$; Fig. 2a; Table S1). There were also no significant effects of bacteria or host on host fecundity ($F_{(1,28)} = 1.38$, $P = 0.25$ and $F_{(1,8)} = 0.01$, $P = 0.91$, respectively), but there was a significant

effect of their interaction ($F_{(1,28)} = 8.06$, $P = 0.008$; Fig. 2b; Table S1). In particular, we found that co-passaged hosts and singly passaged hosts did not differ in terms of survival or fecundity (Student's $t = 0.79$, $P = 0.44$; second vs. third treatment; Fig. 2b). Most surprisingly, co-passaged hosts heat shocked on the ancestral bacteria produced more offspring compared to when they were on their sympatric co-passaged bacteria (Student's $t = 2.84$, $P = 0.008$; Table S2). Similarly, singly passaged, co-passaged, in vitro, and ancestral *B. subtilis* did not differ in how they affected ancestral host fecundity ($F_{(3,14)} = 1.84$, $P = 0.19$; Fig. S1; Table S3), demonstrating that changes in co-passaged bacteria alone were not sufficient to result in decreased host fecundity. Our results overall indicate that changes in both co-passaged hosts and bacteria, and their interaction, were necessary for the observed phenotype.

SPECIFICITY EVOLVED BETWEEN CO-PASSAGED HOSTS AND BACTERIA

We then asked whether the reduced fitness benefit of co-passaged hosts being paired with co-passaged bacteria was due to reciprocal evolutionary change (i.e., coevolution), such that specificity had evolved between hosts and bacteria with shared evolutionary history. Alternatively, lower fitness may be due to copassaging in general, where similar traits have arisen across host and bacterial populations. We tested for local adaptation—a measurement of coevolution—by pairing hosts with their sympatric bacteria or allopatric bacteria and quantifying their fitness after heat
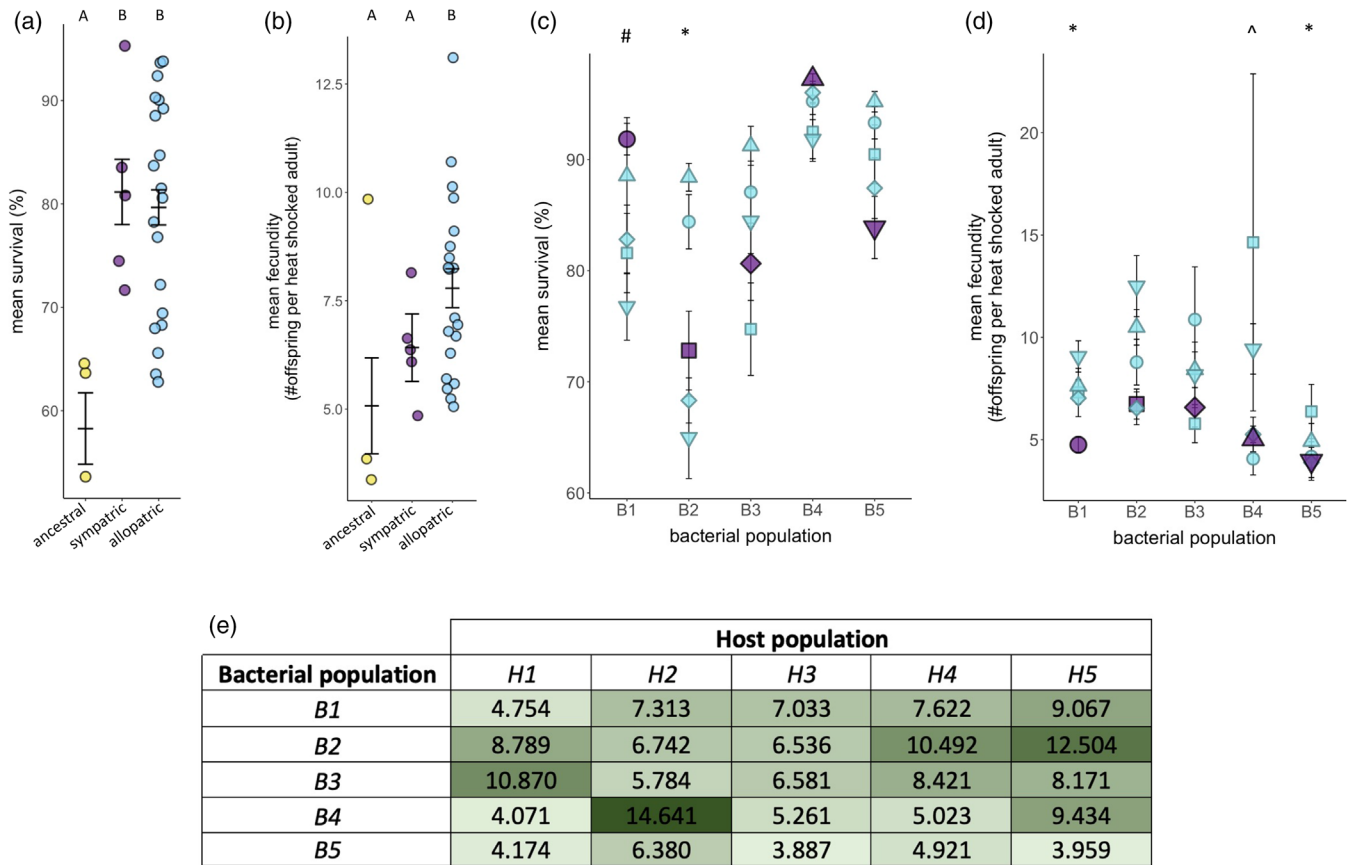
Figure 3. Fitness of co-passaged hosts heat shocked on sympatric versus allopatric bacteria. Co-passaged hosts were heat shocked on the bacteria with which they shared an evolutionary history during experimental evolution (sympatric) or on the other four co-passaged populations (allopatric bacteria). (a) Host survival and (b) host fecundity of ancestral, sympatric, and allopatric combinations. Treatments that are not the same letter are significantly different. (c) Survival and (d) fecundity at the population level. Each data point represents the mean for the indicated host–bacteria combination. $^*P < 0.05$, $^\wedge P = 0.05$, $^\#P = 0.07$. (e) Heat map showing mean fecundity for all combinations of co-passaged host and bacterial populations. Darker colors indicate greater values. The data are combined across three rounds. Error bars indicate standard errors.

shock. We also included the ancestral combination to evaluate changes from the ancestor. We found significant effects of host–bacteria combination on host survival ($F_{(2,81)} = 70.13$, $P < 0.001$; Fig. 3a; Table S4) and host fecundity ($F_{(2,81)} = 43.74$, $P < 0.001$; Fig. 3b; Table S4). Specifically, ancestral hosts paired with ancestral bacteria exhibited lower survival compared to co-passaged hosts (sympatric vs. ancestral: Student's $t = 7.61$, $P < 0.001$; allopatric vs. ancestral: Student's $t = 11.51$, $P < 0.001$). Allopatric combinations produced more offspring than the sympatric or ancestral combinations (Student's $t = 3.44$, $P = 0.009$ and Student's $t = 4.25$, $P < 0.001$, respectively), whereas there was no difference between the sympatric and ancestral combinations (Student's $t = 0.45$, $P = 0.66$). These results demonstrate that although the association is still overall beneficial because sympatric hosts did not decrease in fitness compared to the ancestral pairing (which itself is beneficial under heat shock [Hoang et al. 2019]), they were not able to produce as many offspring when

associated with their sympatric bacteria instead of allopatric bacteria. Next, we assessed the number of bacterial CFUs isolated from sympatric and allopatric host pairings to determine the role of bacterial abundance in the reduction of sympatric pairing fitness. Allopatric pairings resulted in a wide range of CFUs per host and overall there was no statistically significant difference in co-passaged bacterial abundance when paired with their sympatric hosts versus allopatric hosts ($F_{(2,5.8)} = 3.64$, $P = 0.09$; Fig. S2; Table S4). Therefore, the reduction in sympatric host fecundity is likely not directly driven by bacterial density.

CO-PASSAGED BACTERIA CONFER THE LEAST BENEFIT TOWARD THEIR SYMPATRIC HOST

To determine the extent to which specificity and reduced benefits evolved in each population, we proceeded to conduct a similar assay as the previous, this time examining fitness differences at the population (i.e., fine-scale) level. We used a linear contrast

test to compare each sympatric combination against all possible combinations of allopatric pairings involving the focal host and bacterial populations (e.g., H1 with B1 against H1 with B2–B5 and B1 with H2–H5) (Morran et al. 2014; Gibson et al. 2015). Similar to the overall local adaptation results above, co-passaged bacteria conferred the least benefit in terms of fecundity toward their sympatric hosts compared to when they were paired with allopatric hosts (sympatric vs. allopatric: $\chi^2_1 = 13.06$, $P = 0.0003$; Fig. 3d; Tables 1, S5, and S6), whereas there was no significant difference for survival (sympatric vs. allopatric: $\chi^2_1 = 0.08$, $P = 0.78$; Fig. 3c; Tables 1, S5, and S6). Overall, we found that certain combinations of host and bacterial populations resulted in greater fitness gains than others, and that sympatric combinations seldom produced many offspring (Fig. 3e).

Discussion

Coevolution has been shown to be a major contributing factor in shaping mutualistic interactions between hosts and their symbionts across a wide range of symbioses (Suen et al. 2011; Heath et al. 2012; Murfin et al. 2015; Wilson and Duncan 2015; Parker et al. 2017; Gabay et al. 2019; Rekret and Maherali 2019). However, the importance of coevolution in the early stages of nascent associations remains to be elucidated. In this study, we leveraged the amenability of *C. elegans* nematodes to evolution experiments and directly tested how co-passaging of host and bacteria can impact host evolution. We hypothesized that specificity would evolve between partners sharing an evolutionary history, such that fitness gains could be obtained only when hosts were paired with their respective bacteria. Although we found that host–bacteria specificity did arise from co-passaging, these bacteria conferred less fitness benefits toward their sympatric hosts. More specifically, our results suggest that co-passaged hosts had the potential to exhibit greater fecundity after heat stress, but they were impeded from doing so due to association with their bacterial partners.

Our selection regime during experimental evolution involved extracting bacteria from hosts that survived exposure to heat stress and then exposing the next generation of hosts to those bacterial genotypes. Co-passaged bacteria benefitted if their hosts survived heat shock, providing opportunities for both horizontal and vertical transmission to the next generation of hosts. Our system may thus be representative of symbioses in nature where symbionts that are predominantly horizontally transmitted depend on host survival. For example, *Steinernema* nematodes form a mutualism with *Xenorhabdus* bacteria, where both partners depend on each other's survival to parasitize insect hosts. Multiple juvenile nematodes infect the same insect, and thus both vertical and horizontal transmission of *Xenorhabdus* can occur (Goodrich-Blair 2007). Another example is arbuscular mycor-

rhizal fungi, which are horizontally transmitted and obligately dependent on their plant hosts (Raven 2010). In these symbioses, the fungus is reliant on the survival of its host for production of organic carbon. The dependency of these symbionts on the survival of their hosts may thus favor more robust hosts able to withstand environmental stressors, but, from the perspective of the symbiont, there is not necessarily a benefit for increased plant reproduction.

Indeed, symbionts have been shown to improve one aspect of host fitness at the cost of another (Rudgers et al. 2012), and the evolution of mutualism may depend on the improved survival and decreased fecundity of interacting species (Fukui 2014). Within our experiment, co-passaged bacteria improved host survival compared to the ancestral bacteria (Fig. 3a), but there was little incentive for the bacteria to promote host reproduction because the bacteria was not always transmitted directly from mother to offspring. Our findings suggest that the co-passaged bacteria were acting in their own selfish interests, potentially improving host survival at the cost of host reproduction. Singly passaged hosts, by contrast, did not necessarily need to survive for an extended period of time as much as they needed to reproduce to increase their fitness during our experiment. Previous work had indeed found that hosts adapted readily when the bacteria was not evolving (Hoang et al. 2021). If given the chance to evolve first and adapt to the bacteria, the host evolved to gain more benefit from its partner. Thus, coevolving with a new microbial partner in a stressful environment may actually limit the ability of hosts to expand their niche if fitness interests are not aligned.

Previous work between *C. elegans* and its bacterial pathogen, *Serratia marcescens*, found classic patterns of host–parasite specificity, where host mortality was greater when hosts were paired with their sympatric parasite (Morran et al. 2014). We expected the reverse to be true for sympatric, beneficial microbes. Although host and bacteria exhibited specificity in our study, the results were contrary to expectations due to sympatric bacteria being the least beneficial in terms of host reproduction. Furthermore, there was no clear pattern in terms of host survival at the population level (Fig. 3c), suggesting that co-passaging did not have as large an impact on this fitness component. By contrast, two host populations produced significantly more offspring with allopatric bacteria than with sympatric bacteria (a third population was marginally significant), indicating that co-passaged populations are diverging from one another with regard to reproduction (Table 1). Specifically, co-passaged bacteria provided little reproductive benefits toward their sympatric hosts (Fig. 3d), preventing hosts from reaching their evolutionary potential. Indeed, as hosts become trapped in interactions with their bacteria, it may be difficult for them to gain a significant benefit, constraining their ability to adapt to a stressful environment. Because reproduction is a critical component of an organism's fitness, we

Table 1. Fine-scale sympatric versus allopatric test.

Measurement	Sympatric	Allopatric	Chi-square	df	P	Greater survival/fecundity
Survival	B1 and H1	B1 and H2–H5 H1 and B2–B5	3.23	1	0.07	Sympatric (marginally)
	B2 and H2	B2 and H1, H3–H5 H2 and B1, B3–B5	5.76	1	0.02*	Allopatric
	B3 and H3	B3 and H1, H2, H4, H5 H3 and B1, B2, B4, B5	0.89	1	0.35	Neither
	B4 and H4	B4 and H1–H3, H5 H4 and B1–B3, B5	2.34	1	0.13	Neither
	B5 and H5	B5 and H1–H4 H5 and B1–B4	0.12	1	0.73	Neither
Fecundity	B1 and H1	B1 and H2–H5 H1 and B2–B5	4.73	1	0.03	Allopatric
	B2 and H2	B2 and H1, H3–H5 H2 and B1, B3–B5	2.02	1	0.15	Neither
	B3 and H3	B3 and H1, H2, H4, H5 H3 and B1, B2, B4, B5	0.007	1	0.93	Neither
	B4 and H4	B4 and H1–H3, H5 H4 and B1–B3, B5	3.72	1	0.05	Allopatric (marginally)
	B5 and H5	B5 and H1–H4 H5 and B1–B4	7.35	1	0.007	Allopatric

argue that co-passaging led to detrimental effects for the host. In contrast, based on assays of bacterial abundance, we cannot identify a clear beneficial nor detrimental effect for the bacteria, though this measure was conducted at one point in evolutionary time and may not fully capture the dynamic process of coevolution between hosts and bacteria.

We hypothesize that hosts did not maintain or had run out of genetic variation to combat their co-passaged partners and may have reached their short-term adaptive potential. Even though the ancestral host population started with standing genetic variation, it was composed of a low percentage of males. Because sperm is more heat sensitive than oocytes (Gouvêa et al. 2015), we observed little to no males by approximately generation 10 in most experimental evolution treatments. Combined with the bottleneck hosts underwent from repeated heat shock selection, these events would lead to a drastic decrease in host genetic diversity. An influx of genetic variation, such as through gene flow or genetic recombination, may help hosts keep up with their bacteria (Stoy et al. 2020). Although theory suggests that evolutionary rates can affect the evolution of beneficial associations, such that the slower evolving partner obtains more benefits (Bergstrom and Lachmann 2003), our study suggests that, at least for the evolution of a novel beneficial association, evolving quickly could be better for some hosts.

Reciprocal selection between hosts and symbionts has allowed hosts to adapt to diverse conditions, maximizing the benefits that they can obtain from their symbionts (Murfin et al. 2015; Niepoth et al. 2018; Gabay et al. 2019; Rekret and Maherali 2019). From extant symbioses, we can infer that harboring protective microbes would facilitate host adaptation to stressful environments, thus establishing new associations between host and microbe. Previous work found that hosts can adapt to heat stress by being exposed to a nonevolving protective bacterium. Here, we demonstrate that co-passaging of host and microbe does not accelerate host adaptation. Unexpectedly, co-passaging resulted in reduced fitness for hosts paired with their sympatric bacteria, indicating that these bacteria evolved to provide the least benefits toward their partners. Our findings provide direct evidence that coevolution does not have to underlie beneficial associations (Moran and Sloan 2015), and highlight the potential for conflicts to arise between partners. Such conflicts can persist even after a long evolutionary history (Chong and Moran 2016). One way out of this conflict may be for hosts to acquire genetic variation, through sexual recombination or gene flow, for example, in order to respond to their quick evolving partner, allowing them to gain an advantage similar to that of hosts passaged with nonevolving bacteria. Overall, our study sheds light on the fitness consequences for hosts when they are tightly coupled to their bacteria in a nascent interaction, suggesting that reciprocal selection between partners may impede the establishment of novel beneficial associations despite the benefits it brings to established long-term symbioses.

AUTHOR CONTRIBUTIONS

KLH, NMG, and LTM conceived the study and designed the experiments. KLH and HC performed the experiments. KLH and LTM analyzed the data. KLH wrote the manuscript with input from NMG and LTM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

Data are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.v6wwpzgwb.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Fecundity of ancestral hosts on evolved bacteria
Figure S2. Abundance of bacteria in co-passaged hosts.
Table S1. Random effects summary for linear mixed models of co-passaged and singly passaged host fitness on either co-passaged or ancestral *B. subtilis*.
Table S2. Summary of pairwise comparisons between treatments for fecundity of co-passaged and singly passaged hosts on either co-passaged or ancestral *B. subtilis*.
Table S3. Random effects summary for linear mixed model of ancestral host fecundity on evolved and ancestral *B. subtilis*.
Table S4. Random effects summary for linear mixed models of host and bacterial fitness for overall local adaptation.
Table S5. Whole table summary for GLM of host fitness for fine-scale local adaptation tests.
Table S6. Effect tests for GLM of fine-scale local adaptation.