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COEVOLUTION DRIVES TEMPORAL CHANGES IN FITNESS AND DIVERSITY ACROSS ENVIRONMENTS IN A BACTERIA–BACTERIOPHAGE INTERACTION

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Coevolutionary interactions are thought to play a crucial role in diversification of hosts and parasitoids. Furthermore, resource availability has been shown to be a fundamental driver of species diversity. Yet, we still do not have a clear understanding of how resource availability mediates the diversity generated by coevolution between hosts and parasitoids over time. We used experiments with bacteria and bacteriophage to test how resources affect variation in the competitive ability of resistant hosts and temporal patterns of diversity in the host and parasitoid as a result of antagonistic coevolution. Bacteria and bacteriophage coevolved for over 150 bacterial generations under high and low-resource conditions. We measured relative competitive ability of the resistant hosts and phenotypic diversity of hosts and parasitoids after the initial invasion of resistant mutants and again at the end of the experiment. Variation in relative competitive ability of the hosts was both time- and environment-dependent. The diversity of resistant hosts, and the abundance of host-range mutants attacking these phenotypes, differed among environments and changed over time, but the direction of these changes differed between the host and parasitoid. Our results demonstrate that patterns of fitness and diversity resulting from coevolutionary interactions can be highly dynamic.

KEY WORDS: **Bacteria, bacteriophage, competition, fitness costs, genotype-by-environment interactions, host-parasite, productivity, resistance.**

Coevolution between hosts and parasites is thought to play a key role in the maintenance of genetic variation, for instance via the sculpting of defense and counter-defense polymorphisms within natural populations, and to ultimately drive patterns of biodiversity (Thompson 1994; Burdon and Thrall 2000; Buckling and Rainey 2002; Gandon et al. 2002; Little 2002; Nuismer and Otto 2005; Thompson 2005; Kopp and Gavrilets 2006; Tellier and Brown 2007). A number of studies have demonstrated considerable differences among populations in levels of genetic variation in resistance and virulence (Krist et al. 2000; Laine 2005; Springer 2007). This variation in the evolution of resistance and counter-resistance in host–parasite interactions can be driven by spatial and temporal variability in the abiotic environment (Hochberg and van Baalen 1998; Hochberg and Holt 2002; Springer 2007). For example, the environment can strongly influence the strength of parasite-mediated selection (i.e., genotype-by-environment interactions; Gomulkiewicz and Kirkpatrick 1992; Via 1994; Mitchell et al. 2005; Nidelet and Kaltz 2007).

The idea of genotype-by-environment interactions has been extended to explicitly incorporate coevolutionary interactions

in the form of selection mosaics, or genotype-by-genotype-by-environment interactions (i.e., gene in species 1 × gene in species 2 × environment), as a major component of the geographic mosaic theory of coevolution between species (Thompson 1994, 2005; Nuismer 2006; Wade 2007). More specifically, the impact of a particular parasite/host genotype on the fitness of any host/parasite genotype may differ among environments.

Evidence for selection mosaics comes from a diverse range of interactions, including those between crossbills and pines, trematodes and their snail host, *Drosophila* and its parasitoids, *Greya* moths and plants, and salamanders and garter snakes (Kraaijeveld and Godfray 1999; Lively 1999; Benkman et al. 2001; Brodie et al. 2002; Thompson and Cunningham 2002). Just as fitness costs can vary spatially, the fitness of resistant types can also vary across time due to environmental fluctuations, or shifts in the abundance or genetic composition of interacting species (Dybdhal and Lively 1998; Nuismer et al. 2003; Gomulkiewicz et al. 2007). In one environment or at one point in time, resistant types might incur a fitness or competitive cost, whereas in another environment or time point they might not.

This variation in biotic and/or abiotic factors across space and time, and the resulting effects on fitness, should drive patterns of diversity of coevolving species. Ample research provides evidence for differences in abiotic or biotic factors as mechanisms underlying the maintenance of diversity within species and of species richness within communities (Kassen et al. 2004; Hall and Colegrave 2006; Nosil and Crespi 2006; Meyer and Kassen 2007). Productivity (the rate of energy flow in an ecological system) has been shown to be a fundamental driver of diversity, and a number of models predict a unimodal relationship between diversity and productivity (Tilman 1982; Abrams 1995; Rosenzweig 1995; Bohannan and Lenski 2000). This pattern has been empirically demonstrated in multiple studies using microbial systems, which have focused on primary trophic levels (i.e., bacteria; Kassen et al. 2000, 2004; Hall and Colegrave 2006). Nevertheless, we lack a mechanistic understanding of how coevolving interactions can shape phenotypic traits and diversity of hosts and parasitoids across different resource environments and over time.

Here we use a microbial model system, consisting of the bacterium, *Escherichia coli*, and the bacteriophage, T7, to directly test how coevolutionary interactions influence phenotypic variation over multiple generations of coevolution in different resource environments. We were specifically interested in how resource availability affects the competitive ability and diversity of resistant hosts, and the abundance of host-range mutants attacking those hosts, over time. Bacteriophage kill their hosts to reproduce, which imposes strong selection pressure for the evolution of resistance in the host. Bacteriophage then evolve to counter resistance in the host. Bacteria and bacteriophage have proven particularly useful for studies of antagonistic coevolution and provide an ideal model system to test how coevolutionary interactions can drive phenotypic variation and diversity over time and across resource environments (Chao et al. 1977; Lenski and Levin 1985; Shrag and Mittler 1996; Forde et al. 2004, 2007).

We manipulated resource availability (low and high) in a series of experimental microcosms of coevolving *E. coli* and T7 to specifically test the role of resource input in determining the competitive ability and diversity of resistant hosts and the abundance of host-range mutants on the different host phenotypes resulting from an antagonistic coevolutionary interaction. More specifically, we predicted that variation in the competitive ability of resistant hosts should decrease over time due to the action of natural selection, because selection is expected to erode genetic variation in fitness. We also predicted that there should be genotypic differences in competitive ability, which should also be environment-dependent, because the outcome of competition will clearly depend on resource input. We predicted that the diversity of hosts, and the abundance of parasitoids on those hosts, should initially be greater in the high than in the low-resource environment because we previously observed that coevolutionary dynamics occurred at a faster rate in this environment (Forde et al. 2004, 2007). We also predicted that coexistence among types should be transitory, and that changes in abundance over time would likely differ among communities because of the inherent oscillatory dynamics of antagonistic population dynamics and coevolutionary arms races (Seger 1992).

To test our predictions, we measured the competitive ability of resistant hosts relative to the ancestor, the phenotypic diversity of the hosts, and the abundance of parasitoids on those hosts, after the initial invasion of resistant mutants and again after the host and parasitoid had coevolved over approximately 150 bacterial generations in high and low-resource environments. Our results demonstrate significant variation in the competitive ability of resistant hosts as a function of both time and resource availability. Contrary to our initial predictions, both host diversity and parasitoid abundance on the different host phenotypes were greatest in the low-resource environment at the start of the experiment. However, the number of different bacterial phenotypes decreased over time in the high-resource environment, but increased in the low-resource environment. In contrast, the abundance of host-range mutants on these phenotypes decreased over time in the low-resource environment and increased over time in the high-resource environment. Our results illustrate how patterns of phenotypic diversity, driven by differences in resource availability, change over time due to the dynamical nature of the coevolutionary arms race.

Methods

STUDY SYSTEM

The coevolutionary dynamics of *E. coli* and T7 can broadly be divided into a series of phenotypic classes based on the sequential

emergence of patterns of resistance and counter-resistance (Chao et al. 1977). Ancestral, T7-sensitive bacteria (B_0) first evolve resistance to ancestral phage ($T7_0$), after which these new strains are referred to as first-order resistant bacteria (B_1). T7 can then evolve to attack the first-order-resistant bacteria and are referred to as host-range mutants ($T7_1$), which can also attack B_0. Second-order resistant bacteria (B_2) that are resistant to $T7_0$ and $T7_1$ can also arise and eventually invade the community. However, as we demonstrate below, there is considerable phenotypic diversity underlying these discrete classes of resistance in the host.

Coevolution between *E. coli* and the bacteriophage T7 is mediated through the ability of the bacteriophage to penetrate the bacterial cell, and the ability of bacteria to prevent penetration by bacteriophage. The cell envelope of *E. coli* is composed of three layers: the outer membrane, the peptidoglycan, and the inner membrane. The outer membrane is composed of proteins, lipids, and lipopolysaccharides (LPS). LPS are found almost entirely within the external portion of the outer membrane (Laird et al. 1994). LPS serve as an important component of the permeability barrier of the cell and are involved in cell wall stability (Qimron et al. 2006). Furthermore, bacteriophage T7 use LPS as receptors for attachment to the cell, and LPS are involved in subsequent stages of bacteriophage adsorption, including serving as a trigger for ejection of DNA (Tamaki et al. 1971).

Resistance to T7 by *E. coli* B is conferred through mutations that truncate LPS, resulting in loss of the receptor moiety to which T7 binds and thereby preventing adsorption of the virus (Tamaki et al. 1971; Lenski 1988). This truncation can be shallow or deep within the LPS structure, depending on the actual mutation (Sen and Nikaido 1991). The nature of the truncation can have pleiotropic effects on outer membrane chemistry, most notably the trimerization of porins. Deep truncation tends to reduce the number of functional porin multimers in the outer membrane (i.e., outer membrane proteins, OMPs; Sen and Nikaido 1991), which can lower fitness. OMPs form water-filled channels that allow diffusion of nutrients into the cell, and changes in the structure or number of these channels could impose a fitness cost via impairment of nutrient uptake. The important roles of LPS and OMPs in bacterial physiology provide a mechanistic basis for a potential trade-off between bacterial growth rates and resistance to the bacteriophage, which could affect competitive ability relative to the ancestor.

EXPERIMENT

Communities of hosts and parasitoids were initially established through inoculation of isogenic strains of *E. coli* (strain REL607, Lenski et al. 1991) and of T7 (obtained from the American Type Culture Collection) into chemostats (30 mL communities run at a dilution rate of 0.2 turnovers/h). Two types of communities were established by manipulating the limiting nutrients for the bacteria: high resource (1000 μg/mL of glucose; three replicates) and low resource (10 μg/mL glucose; two replicates). T7-resistant colonies and samples of the phage population were isolated after the initial invasion of the resistant mutants (day 3) and after the host and parasitoid coevolved for over 150 bacterial generations (day 17) of the experiment. Average populations sizes in the high and low-resource environments, respectively, on each day were as follows: day 3, bacteria $= 3.3 \times 10^4$ and 1.1×10^3, phage $= 5.3 \times 10^6$ and 2.6×10^6; day 17, bacteria $= 1.1 \times 10^5$ and 1.6×10^4, phage $= 9.6 \times 10^4$ and 7×10^4.

Variation in the competitive ability of resistant hosts

We were interested in whether there was significant variation in phenotypic traits of T7-resistant hosts from the low- and high-resource environments, and if the amount of variation in these traits changed over time. To address this, we measured the competitive ability of the resistant hosts relative to the ancestral strain. Resistant colonies were isolated from the chemostat samples by taking approximately 10 μl from each replicate, plating it with 50 μl of the ancestral strain of T7 on agar plates (titer of approximately 7×10^8), and incubating the combined sample at 37°C overnight. Between four and eight colonies from each replicate plate were then randomly selected. Hence, the total number of independent colonies for each resource level and each time point ranged between 10 and 20. Each colony was then streaked on an agar plate to remove any residual T7 present in the cells, and grown overnight in the same type of liquid medium as used in the original experiment (i.e., either high or low resources). Freezer stocks of each culture were then stored in glycerol at −80°C for future use.

Phenotypic variation in competitive ability of the T7-resistant mutants was determined through competition with an ancestral strain carried out in the same medium used in the original experiment (i.e., high or low resources). T7 resistance in the bacteria was confirmed prior to the competition experiment by streaking a portion of each culture across a 20 μl aliquot of the ancestral strains of T7 dried on an agar plate.

Competition assays were performed as described in Lenski et al. (1991). In these assays, the absolute growth rate of one genotype over time depends on the presence and abundance of the other genotype. We conducted the assays in batch culture. Although relative competitive ability measured in batch culture may differ from that measured in chemostat culture (Bohannan et al. 2002), we were interested primarily in a measure of phenotypic variation, rather than a direct measure of fitness in the experimental environment. Chemostat measures of competitive ability are much more difficult, time-consuming and resource-intensive than those in batch culture, and we would not have been able to assay a sufficient number of isolates had we used chemostat measures.

Both competitors were inoculated separately from the freezer stock into 9.9 mL of the appropriate media and allowed to grow overnight. One hundred microliters of each culture were transferred to 9.9 mL of fresh media the next day, and the two strains were allowed to grow for another 24 h. This procedure helped to ensure that the starting densities of the two competitors were similar, as the number of cells in the initial ice sample from the freezer stock can be variable. Fifty microliters of each competitor were then added to 9.9 mL of medium and allowed to grow together for 24 h. The densities of each competitor were determined at the start (after mixing) and end of the 24-h period. Evolved and ancestral competitors were distinguished by the neutral Ara marker, which denotes the ability to take up L(+)arabinose and results in a color difference (pink vs. red) between the two strains (Lenski et al. 1991).

Relative competitive ability was calculated based on the average per capita rate of increase of each strain over 24 h (t_0 to t_1)

$$W_{ij} = \frac{\ln(N_i(t_1)/N_i(t_0))}{\ln(N_j(t_1)/N_j(t_0))},$$

where N_i and N_j are the densities of the evolved and ancestral strains, respectively.

Individual assays were performed for 19 colonies from the high and 12 colonies from the low-resource communities from day 3, and 11 colonies from the high and 14 from the low-resource communities from day 17 of the original experiment. These numbers were based on the maximum number of colonies that could be grown up overnight repeatedly without reverting to T7 sensitivity. Each assay was also replicated two to three times (totaling 106 assays) and the competition values for each colony were averaged.

Genotype-by-environment interactions

Competition assays were also performed on isolates from the start of the experiment in the alternate resource level (i.e., low-resource colonies were assayed in high-resource medium) to assess if genotype-by-environment interactions in this phenotypic trait were present. A total of 13 colonies from the high-resource environment and 10 colonies from the low-resource environment were assayed in reciprocal resources. These numbers were based on the maximum number of colonies that could be grown up reliably in the alternate resource environment. Each assay was replicated twice.

Phenotypic diversity of resistant hosts

We further evaluated the phenotypic diversity associated with T7 resistance in the bacteria by assaying sensitivity to the antibiotic novobiocin and to a series of bacteriophage. The same colonies used for the fitness assays were used to determine phenotypic diversity of the hosts. However, resistance, partial resistance, and/or sensitivity to the phenotypic assays could not be reliably determined for two colonies from the low-resource communities on days 3 and 17.

Table 1. Bacteriophage screen used to determine phenotypic diversity in the T7-resistant hosts. The antibiotic novobiocin was also used in the assays (s = sensitive, pr = partially resistant, and r = resistant).

Phenotype	Novobiocin	T4	T2	Tu1a	K3
1	s	r	s	pr	s
2	s	r	s	s	s
3	pr	r	s	s	s
4	pr	r	s	pr	s
5	s	r	s	r	s
6	s	pr	s	r	s
7	s	r	pr	r	pr
8	s	r	pr	r	s
9	pr	r	pr	r	s
10	pr	r	s	s	s
11	pr	r	r	r	r

Mutants with truncated LPS are sensitive to the antibiotic novobiocin (Tamaki et al. 1971). Ten microliters from an overnight culture of each strain was added to 1 mL of media with 1000 μg/mL of glucose both with novobiocin added at a concentration of 125 μg/mL and without novobiocin. The sensitive, ancestral strain was assayed as a control. Cultures were grown overnight at 37°C. Resistance, partial resistance, or sensitivity to the antibiotic was evaluated by comparing the turbidity of the cultures in the presence and absence of the antibiotic.

Mutants were further evaluated using a series of bacteriophage screens to determine phenotypic diversity (Table 1). Resistance to the bacteriophage T4 occurs through mutations that cause defects in the LPS core of the host membrane and can cause cross resistance to T7 (Demerec and Fano 1945). Resistance to T2 indicates that the LPS mutation has also affected the assembly or function of OmpF. T2 uses both a part of LPS distal to the T7 receptor and OmpF to infect *E. coli* cells (Lenski 1984). Resistance to Tu1a also indicates effects on OmpF (Schwartz 1980). OmpF is the most common membrane protein in *E. coli* and is involved in osmoregulation and nutrient uptake (Datta et al. 1977; Travisano and Lenski 1996). Resistance to K3 indicates that the truncation of LPS has also affected the assembly or function of OmpA (Schwartz 1990). OmpA is involved in amino acid transport and the structural integrity of the outer membrane (Marona et al. 1985; Heller 1992).

Twenty microliters of each bacteriophage was dried on an agar plate and each bacterial isolate was streaked across the bacteriophage to assess resistance. A total of 11 phenotypes were assigned to the bacteria based on whether individual colonies were

sensitive, partially resistant, or resistant to novobiocin and each bacteriophage (Table 1).

Growth kinetics

The growth curves for the T7-resistant mutants used to determine phenotypic diversity were measured when grown in isolation to evaluate if the phenotype differentially affected growth kinetics, and if the growth kinetics could be used to understand patterns of phenotypic diversity. These assays were performed in 96-well microtiter plates using a SpectraMax M2e microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Three replicate growth curves were obtained for each phenotype.

Before measuring growth kinetics, all bacterial isolates were grown in the appropriate medium (high resource: 1000 μg/mL or low resource: 10 μg/mL resource). High-resource isolates were grown up overnight and low-resource isolates were grown for an additional 24 h to achieve adequate cell densities. T7 resistance was confirmed as described above. Five microliters of the overnight culture of each high-resource isolate were transferred to 200 μl of 1000 μg/mL glucose media in a flat bottom microtiter plate. Twenty microliters of the overnight culture of each low-resource isolate were transferred into 180 μl of 10 μg/mL glucose media in a microtiter plate.

All plates were incubated at 37°C and shaken for 15 min every 30 min. Optical density (OD) was measured at a wavelength of 600 nm every 30 min for approximately 10 h. Replicate values of OD for each isolate were averaged, and then the average OD values for each phenotype were plotted over time. OD is directly proportional to population size. Differences in growth kinetics were determined by evaluating the amount of overlap among error bars.

Abundance of phage on different bacterial phenotypes

We determined the abundance of host-range mutants on different bacterial types present in the communities by adding 30 μl of chloroform to 1500 μl of a sample taken from each community and vortexing the mixture to kill any bacteria that were present. One hundred microliters of each sample of the phage population were plated on a lawn of each bacterial isolate to determine the abundance of phage on each of the host phenotypes (1–11). Phage were plated on bacterial isolates from the same chemostat from which they originated. We used the "efficiency of plating" (EOP; the number of plaques on each host, Forde et al. 2004) as a measure of phage abundance.

DATA ANALYSIS

All data were checked for normality and homogeneity of variances prior to analyses and met assumptions required for analyses.

Variation in the competitive ability of resistant hosts

Differences in the variance in relative competitive ability of colonies from each community and time point were tested using F-tests (Sokal and Rohlf 1995).

Genotype-by-environment interactions

Genotype-by-environment interactions were tested using a two-way analysis of variance (ANOVA) with assay resource and colony as the main effects. Colony was treated as a random factor and nested within chemostat.

Phenotypic diversity of resistant hosts

We tested for differences in the frequencies of phenotypes over time and in the low and high-resource environments based on the 11 phenotypes in Table 1. Samples were combined to evaluate differences in the frequencies of phenotypes across all populations, as described above. A log-linear model was used to test for significant differences in novel phenotype frequencies in the low- and high-resource communities on each day, as well as an interaction between resource level and day (Sokal and Rohlf 1995).

Abundance of phage on different bacterial phenotypes

We tested for differences in the abundance of host-range mutants on the bacterial phenotypes in the low- and high-resource communities on each day, and for interactions between resource level, host phenotype, and day, using ANOVA.

Results

VARIATION IN THE COMPETITIVE ABILITY OF RESISTANT HOSTS

Variation in competitive ability of resistant hosts relative to the ancestor decreased over time for colonies from both environments. Variation in relative competitive ability of colonies from both the low and high-resource environments was approximately twice as great on day 3 as on day 17. This decrease over time was statistically significant for colonies from the high-resource community ($F_{1,2} = 19.22$, $P < 0.05$), suggesting a possible greater rate of erosion of variance in fitness in these environments.

GENOTYPE-BY-ENVIRONMENT INTERACTIONS

At the beginning of the experiment, the relative competitive ability of high-resource isolates varied with resource levels, as indicated by the significant genotype-by-environment interaction (Fig. 1; Table 2). In contrast, the genotype-by-environment interaction was not significant for low-resource isolates, although the rank order of fitness did change between resource levels. For some isolates, relative competitive ability was lower in the low-resource environment than in the high-resource environment, whereas for others, the reverse was true. For some isolates, there was no effect

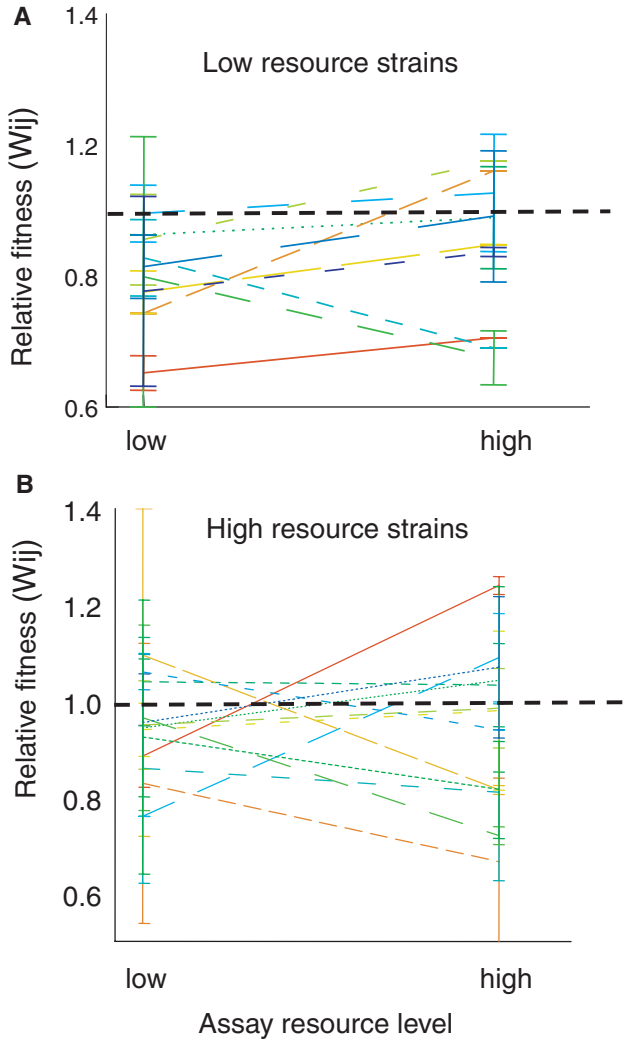

Figure 1. **(A) Relative fitness of resistant colonies on day 3 from the low-resource communities, assayed in low and high-resource environments (±SE). (B) Relative fitness of resistant colonies on day 3 from the high-resource communities, assayed in low and high-resource environments (±SE). The dashed lines at 1 indicate equal fitness of the ancestor and evolved strain.**

of resource level on competitive ability. There were significant differences in competitive ability among isolates in both the high and low-resource communities (Table 2), and the competitive ability of individual isolates was often below one, suggesting a cost of resistance (Fig. 1). The range (max to min) of relative fitnesses was also greater for the isolates drawn from the high-resource environment, when measured in each environment.

PHENOTYPIC DIVERSITY OF RESISTANT HOSTS

The frequencies of phenotypes varied over time and between resource levels (Fig. 2A, Table 3). Similar numbers of phenotypes were present in each community at the start of the experiment (5 in low, 4 in high), three of which were the same phenotypes (types 1, 2, and 5). However, the difference in the proportions of

Table 2. **Results from a two-way analysis of variance of the effects of assay resource (fixed) and colony (random) on fitness. Colony was nested within chemostat.**

Source	df	*F*-ratio	*P*-value
Low-resource environment			
Assay resource level	1	2.15	0.157
Colony (chemostat)	9	4.367	0.002
Assay resource × colony (chemostat)	9	0.967	0.492
Error	22		
High-resource environment			
Assay resource level	1	0.004	0.949
Colony (chemostat)	12	2.999	0.007
Assay resource × colony (chemostat)	12	2.943	0.008
Error	31		

phenotypes between resource levels increased over time, and only phenotype 1 was found in both communities at the end of the experiment. Furthermore, over twice as many phenotypes occurred in the low-resource communities as in the high-resource communities. There was thus divergence in genotypic composition as a function of resource level, and genetic variation was maintained more effectively in the low-resource environment.

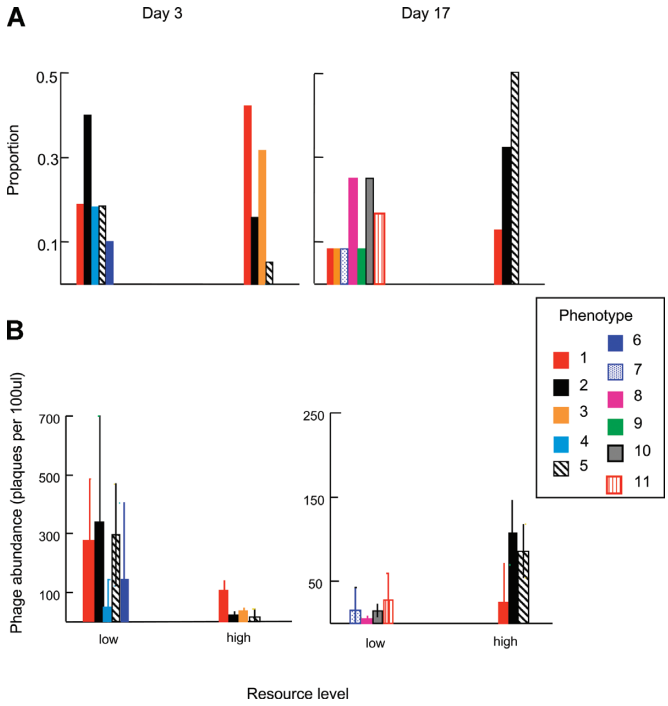

Figure 2. **(A) Proportions of different bacterial types on day 3 and day 17 from the low and high-resource communities. (B) The abundance of host-range mutants on the different bacterial phenotypes from low and high-resource environments on days 3 and 17.**

Table 3. Results from a log-linear model of frequency of phenotypes as a function of day and resource level.

Term	chi-square	Df	*P*-value
Resource level	0.95	1	0.33
Phenotype	18.72	10	0.04
Day	0.004	1	0.95
Resource × phenotype	14.40	10	0.16
Resource × day	0.07	1	0.80
Day × phenotype	16.96	10	0.075

GROWTH KINETICS

The growth kinetics of different phenotypes can be used to understand patterns of phenotypic diversity in each resource environment and over time. There were no differences in the growth kinetics among phenotypes from the low-resource environments from either day 3 or 17, which may have facilitated the coexistence of the different phenotypes in these communities at the end of the experiment (Fig. 3A; Chesson 2000). In contrast, type 5 from the high-resource community on day 3 grew to the highest OD of all phenotypes (Fig. 3B), and dominated the community on day 17 (Fig. 2A).

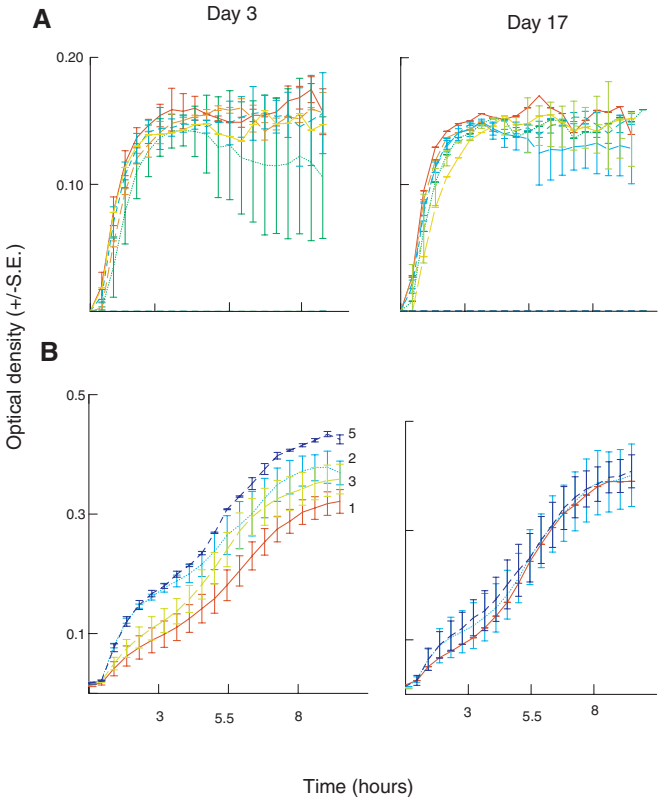

Figure 3. Growth kinetics of different phenotypes from the (A) low-resource communities and (B) high-resource communities on day 3 and day 17. Numbers next to the lines indicate the different phenotypes.

Table 4. Results of the analysis of variance of phage abundance as a function of bacterial phenotype, day, and resource level.

Source	df	*F*-ratio	*P*-value
Resource level	1	3.144	0.081
Phenotype	10	2.937	0.004
Day	1	6.255	0.015
Resource × phenotype	10	0.958	0.489
Resource × day	1	9.723	0.003
Day × phenotype	10	1.625	0.121
Resource × phenotype × day	10	2.147	0.034
Error	62		

ABUNDANCE OF PHAGE ON DIFFERENT BACTERIAL PHENOTYPES

The abundance of host-range mutants on the bacterial phenotypes differed among host types, resource levels, and changed over time (Table 4, Fig. 2B). All values shown are much lower than the total virus titer. At the start of the experiment, the abundance of phage originating from the low-resource communities was greatest on host types 1, 2, and 5, on average. Furthermore, phage abundance was initially greater overall on the bacterial phenotypes from the low-resource communities compared to abundance on hosts from the high-resource communities. In the high-resource environment, the abundance of phage was greatest on host type 1. This pattern changed and indeed was reversed by the end of the experiment. On day 17, the abundance of host-range mutants originating from the low-resource environments was much lower than that of phage originating from the high-resource environment. In the high-resource community, the abundance of phage was greatest on host types 2 and 3, and the abundance of phage on host type 1 was lowest, on average.

Discussion

Our results suggest that there can be significant variation in the phenotypes of resistant hosts, and that this variation is both time- and environment-dependent. More specifically, there was marked variation in both competitive ability and diversity of the resistant hosts resulting from coevolution with the parasitoid, and there appears to be some costs to resistance. Furthermore, there were differences in the abundance of parasitoids on those hosts in the different resource environments and over time.

VARIATION IN THE COMPETITIVE ABILITY OF RESISTANT HOSTS

The action of natural selection is expected to decrease fitness variation among genotypes and phenotypes over time. We found that variation in the competitive ability of T7-resistant hosts relative to the ancestral strain decreased between the start and end of our experiment, likely due to the selection pressures imposed through

coevolution with the bacteriophage. Interestingly, there was no difference in the variation in relative competitive ability between the low- and high-resource environments. One might expect that variation would be greater in the high-resource environment, potentially due to decreased competition for available nutrients relative to that in the low-resource environment. However, in the high-resource environment, there was also a higher abundance of bacteria, so the intensity of competition may not have so strongly differed between the two environments. This variation in competitive ability was further illustrated by the genotype-by-environment interactions evident in our study.

GENOTYPE-BY-GENOTYPE-BY-ENVIRONMENT INTERACTIONS

Genotype-by-environment interactions often maintain genetic variation within and among populations (Gomulkiewicz and Kirkpatrick 1992; Via 1994; Mitchell et al. 2005; Nidelet and Kaltz 2007). Past work on microbial taxa has found significant interactive effects among mutation-type, temperature, and resource (maltose or glucose) on relative fitness among *E. coli* mutants (Remold and Lenski 2001). Susceptibility of *Daphnia* clones to bacterial parasites (Mitchell et al. 2005) and the susceptibility of pea aphids to fungal pathogens (Blanford et al. 2003) can also differ under varying temperatures. In our system, genotype-by-environment interactions stem from coevolutionary interaction with the bacteriophage. Some colonies showed reduced competitive ability whereas others did not, and this difference depended on resource levels. Thus, these results represent more complex genotype-by-genotype-by-environment interactions, giving rise to the potential for selection mosaics at landscape and geographical scales (Thompson 1994, 2005; Nuismer 2006; Wade 2007). Patterns of relative competitive ability among bacterial isolates likely reflect environment-dependent effects of parasite genotypes on the hosts.

PHENOTYPIC DIVERSITY AND GROWTH KINETICS OF THE HOSTS

The number of different bacterial phenotypes was greater in the low-resource environments at both the start and end of the experiment. It increased over time in the low-resource communities, but decreased over time in the high-resource communities. Brockhurst et al. (2004) and Benmayor et al. (2008) also found that phage increased the diversity of *Pseudomonas fluorescens* in low-productivity environments, likely due to selection for phage-resistant variants under low-resource situations. Phage increased diversity in less-stressful environments, it is hypothesized through reduced competition for resources (Benmayor et al. 2008). Thus, our results can be generalized to at least some other microbial systems. In general, a positive effect of phage on diversity is particularly likely if they are relatively specialized in their impact upon bacterial strains (Levin et al. 1977), for this permits phage to reduce interspecific competition more than intraspecific competition, which is needed for any environmental factor to facilitate competitive diversity (Chesson 2000; Chase et al. 2002).

Differences in the growth kinetics among mutants may explain these differences between high- and low-growth environments. No differences occurred in the growth kinetics among low-resource phenotypes, and this similarity among types likely underlies the coexistence of multiple different types at this resource level. Chesson (2000) refers to such similarities as an "equalizing factor," which can permit subtle niche differences to suffice for coexistence, or lead to very long-term transient coexistence. In other words, no one type dominated these communities at the time scale of our experiments. In contrast, the abundance of type 5 increased between the beginning and end of the experiment in the high-resource communities, and this type reached the highest OD of isolates assayed from day 3 (Fig. 3B). Thus, this phenotype may have been the dominant competitor in the community. Higher growth rates may lead to shorter transient phases of coexistence; high resources may increase the scope for kinetic differences among strains to be expressed, thus enhancing competition and reduction in genetic diversity. We note that Figure 3 also suggests other aspects of emergent heterogeneity due to differences in resource supply. The phenotypes present in the low-resource environment asymptoted to carrying capacity quite rapidly, whereas for those phenotypes present in the high-resource environment there is a tendency for the initial growth to be slower, but the final density to be higher than observed for isolates drawn from the low-resource environment. Moreover, the growth curves are overall more similar within resource treatments, than between them. This too may be an indication of the importance of fitness equalization as a factor maintaining genetic diversity within each environment.

The relationship between diversity and resource levels is thus likely to depend on the absolute levels of productivity. Envision a typical unimodal relationship between productivity and diversity. At very high levels of productivity, diversity will be low because one or a few types outcompete all others and dominate the community. This is consistent with our results: phenotype 5 grew to the highest OD and was in greatest abundance in the high-resource communities. At lower levels of productivity, the number of different types can be greater, as seen in our results, due the coexistence of these multiple types. However, at even lower resource levels than used in these experiments, diversity is likely to again be low, because total numbers are low, and when dividing this low number among several genotypes drift can readily lead to a loss of diversity because of random walks to extinction. In future work, it would be interesting to run experiments such as these over much longer time spans, to assess if the differences in diversity are maintained (e.g., because of stabilizing, frequency-dependent selection).

THE ABUNDANCE OF HOST-RANGE MUTANTS OF THE DIFFERENT BACTERIAL PHENOTYPES

At the beginning of the experiment, the abundance of host-range mutants on the different bacterial phenotypes was lower, on average, in the high than in the low-resource environment. However, this pattern reversed after over two weeks of coevolution with the host; the abundance of host range mutants was greater in the high-resource environment than in the low-resource environment. This pattern contrasts with that seen in the host, where the number of different bacterial phenotypes increased over time in the low-resource environment, but decreased over time in the high-resource environment. Thus, the effects of resource availability on patterns of diversity (i.e., polymorphisms within or among types) may differ between trophic levels. Furthermore, our results illustrate the importance of explicitly addressing temporal dynamics in studies of coevolution, and the need to articulate how such temporal dynamics is environment-dependent.

The genetic diversity of species is shaped by evolutionary and coevolutionary processes acting at different rates and in different ways in different environments. We found that under low-resource levels, the number of different resistant host types increased after coevolution with the parasitoid. In contrast, host diversity decreased over time in the high-resource environment. However, the abundance of host-range mutants on these bacterial phenotypes showed the opposite pattern: abundances decreased over time in the low-resource environment and increased over time in the high-resource environments. Thus, we caution that relationships between diversity and productivity may prove to be transient in many systems, and depend on the trophic level of interest. Our results illustrate that patterns of diversity in coevolving microbial species are context-dependent; specifically, resource levels can mediate the effects of host–parasitoid coevolution on phenotypes. Also, our results show that these patterns can change over time due to the dynamical nature of the coevolutionary interaction within and across complex environments, pointing to the existence of complex selection mosaics within coevolving interactions.

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