Evolutionary ecology of microorganisms: from the tamed to the wild

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ABSTRACT

An overarching goal of biology is to understand how evolutionary and ecological processes generate and maintain biodiversity. While evolutionary biologists interested in biodiversity tend to focus on the mechanisms controlling rates of evolution and how this influences the phylogenetic relationship among species, ecologists attempt to explain the distribution and abundance of taxa based upon interactions among species and their environment. Recently, a more concerted effort has been made to integrate some of the theoretical and empirical approaches from the fields of ecology and evolutionary biology. This integration has been motivated in part by the growing evidence that evolution can happen on "rapid" or contemporary time scales, suggesting that eco-evolutionary feedbacks can alter system dynamics in ways that cannot be predicted based on ecological principles alone. As such, it may be inappropriate to ignore evolutionary processes when attempting to understand ecological phenomena in natural and managed ecosystems. In this chapter, we highlight why it is particularly important to consider eco-evolutionary feedbacks for microbial populations. We emphasize some of the major processes that are thought to influence the strength of eco-evolutionary dynamics, provide an overview of methods used to quantify the relative importance of ecology and evolution, and showcase the importance of considering evolution in a community context and how this may influence the dynamics and stability of microbial systems under novel environmental conditions.

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HIGHLIGHTS

- Evolutionary processes can occur on "rapid" or contemporary time scales
- Rapid evolution may be particularly important for understanding dynamics of microbial systems
- Evolutionary change can influence ecological processes, which can result in feedbacks that influence system behavior

A. Overview: interplay between ecological and evolutionary processes

An overarching goal of biology is to understand how evolutionary and ecological processes generate and maintain biodiversity. Despite this seemingly unified goal, historically, the fields of evolutionary biology and ecology have largely advanced in isolation of one another. While evolutionary biologists interested in biodiversity tend to focus on the mechanisms controlling rates of evolution and how this influences the phylogenetic relationship among species, ecologists attempt to explain the distribution and abundance of taxa based upon interactions among species and their environment. Recently, a more concerted effort has been made to integrate some of the theoretical and empirical approaches from the fields of ecology and evolutionary biology. This integration has been motivated in part by the growing evidence that evolution can happen on "rapid" or contemporary time scales (1). When this occurs, evolutionary changes can select for functional traits and behaviors of species in ways that influence ecological processes, such as population dynamics, the outcome of species interactions, and even ecosystem functioning (2-5). Ultimately, eco-evolutionary feedbacks can alter system dynamics in ways that cannot be predicted based on ecological principles alone (6) (Fig. 1). As

such, it may be inappropriate to ignore evolutionary processes when attempting to understand ecological phenomena in natural and managed ecosystems.

Evolutionary ecology is a broad discipline that covers a wide range of topics including life history theory, sexual selection, sociobiology, and co-evolution, which are addressed in greater detail elsewhere (7-9). In this chapter, we highlight questions and approaches that are relevant to studying the evolutionary ecology of microorganisms, with a focus on rapid evolution. Because there is no single "right" way for conducting research on the evolutionary ecology of microorganisms, we provide an overview of some of the commonly used methods used in experimental evolution, along with studies that track evolution in the wild using sequencing-based approaches. We emphasize some of the major processes that are thought to influence the strength of eco-evolutionary dynamics, provide an overview of methods used to quantify the relative importance of ecology and evolution, and showcase the importance of considering evolution in a community context and how this may influence the dynamics and stability of microbial systems under novel environmental conditions.

B. Why study the evolutionary ecology of microorganisms?

While textbooks dealing with evolution and ecology tend to highlight macroscopic organisms (e.g., insects, plants, and fish) there are a number of important reasons why scientists should consider the evolutionary ecology of microbes:

1) Microorganisms are diverse – Microorganisms comprise the vast majority of the planet's biodiversity. Owing to recent advances in sequencing technology, we now know that most phyla in the tree of life are comprised of microbial taxa. At local scales, the richness (a primary component of α–diversity) of microbial taxa within a given a given habitat (e.g., soils,

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ocean, gut) can be quite high. It is not uncommon to recover thousands of bacterial "species" from a single sample (10). In addition, there is high compositional turnover (i.e., β-diversity) of microbial communities in both time and space (11, 12). By convention, most scientists study the diversity of bacterial and archaeal communities using operational taxonomic units (OTU), which are based on comparative analysis of 16S rRNA gene sequences. Populations whose 16S rRNA sequences are > 97% similar are considered to be members of the same taxon. Although this similarity cutoff correlates well with DNA-DNA reassociation kinetics that are used to define microbial species (13, 14), it underestimates the extensive microdiversity that is commonly found within various groups of microorganisms (15-17). Collectively, the standing genetic and phenotypic variation found in microbial communities provides a plethora of materials for ecological and evolutionary processes to act upon.

2) Microbes have high evolutionary potential – Owing to their large population sizes and short generation times, microorganisms have the potential to evolve much faster than plants and animals. In addition, microbes tend to live in close proximity with one another (e.g., biofilms), which allows them to share resources, byproducts, and establish co-evolved, syntrophic interactions (18). In theory, the lack of sexual reproduction should drastically reduce rates of evolution in species with finite population sizes, since sex, through recombination, accelerates the rate at which multiple favorable mutations emerge within a genome (19). However, homologous recombination occurs within and between microbial populations (20, 21) and microorganisms can acquire novel sources of genetic information through horizontal gene transfer (22). Even at low frequencies, the vast population size of microbes ensures that such mechanisms, in combination with mutation, generate large reservoirs of diversity for evolutionary processes to act upon.

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3) Microbes are model systems for studying evolutionary ecology – Compared to "macrobial" systems, microorganisms have unique features that can readily be harnessed for studying evolutionary ecology (23). With microorganisms, one does not typically need to be concerned about small population sizes, which can be important when making inferences about evolutionary processes. Moreover, many of the taxa that are used in laboratory settings have fairly short generation times, which is a requisite for studying "evolution in action". While great progress has been made in evolutionary ecology by studying model organisms (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*), an increasing number of microorganisms can be isolated from the natural environment and maintained under laboratory conditions (24, 25). In some cases, these microbes are amenable to genetic manipulation, which means that scientists can explore the genetic underpinnings of phenotypic traits using molecular tools such as recombineering. In microbial systems where genetic manipulations are not feasible, scientists are taking advantage of advances in genomics, transcriptomics, proteomics, and metabolomics to explore the eco-evolutionary complexities of microbial communities (26-28). Together, these features allow evolutionary ecologists to explore gene-gene interactions (e.g., epistasis) along with fitness trade-offs that tend to influence the strength of natural selection in different environments.

C. A traits-based approach to the evolutionary ecology of microorganisms

One of the most important criteria for studying evolutionary ecology is the ability to identify and quantify changes in the functional traits of a focal population. Functional traits can be defined as morphological, behavioral, or physiological properties that influence the fitness of an individual under a given set of conditions (29). These properties have a genetic basis and are

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passed down from one generation to the next (i.e., they are heritable). Measuring traits can be fairly straightforward for some biologists. For instance, in the textbook example of Darwin's finches, the relative frequency of beak sizes changes through time as a function of precipitation variability and the resulting distribution of seed sizes (30). In principle, similar approaches can be applied to microorganisms.

Quantifying traits that are under natural selection can be challenging when studying microorganisms. Often the morphological characteristics among divergent taxa, observed using standard microscopy, appear identical. Other traits, such as metabolic functions, can be measured under laboratory conditions, but it is difficult to cultivate the vast majority of microorganisms from natural environments. Consequently, there are hurdles to studying the evolutionary ecology for most of the life on our planet. However, there is a growing set of tools that can be used for studying microbial traits. For example, it is now possible to visualize traits of individuals, such as the capacity for nitrogen fixation, using high-resolution nanometer scale secondary ion mass spectrometry (NanoSIMS) (31) or single-cell resource quotas using Raman microspectroscopy (32). Similarly, the chemotactic behavior of bacteria in relation to resource patches can be observed using a combination of microfluidics and advanced image analysis (33).

Genotypic features (a.k.a "genotypic traits") provide a novel opportunity and potentially transformative way of characterizing microbial traits (34). Although it is well established that genetic information does not always translate directly into an observable phenotype, the presence or absence of, for example, a *nifH* gene will help predict whether or not an organism has the capacity to carry out nitrogen fixation. One of the most commonly used high-throughput methods to date involves marker gene analysis of the small subunit rRNA gene. This type of an approach characterizes the phylogenetic diversity of a microbial sample in a cost-effective way.

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In some cases phylogenetic gene markers can be a good proxy for functional traits, but this is
162 determined by the degree to which a trait of interest is phylogenetically conserved (35). Recent studies suggest that phylogenetic conservation in bacteria and archaea depends on trait
164 complexity, with simpler traits (e.g., glucose utilization) being more phylogenetically dispersed than complex traits (e.g., methanogenesis) (36).

166 However, we are no longer restricted to making inferences about microbial traits based on a single gene. For example, whole genomes are now being used to gain eco-evolutionary
168 insight into the lifestyles of cultivated organisms (37). Furthermore, we are increasingly able to identify relevant genotypic traits using cultivation independent approaches that rely on gene
170 inventories and their expression patterns derived from nucleic acids (DNA and RNA) and proteins extracted from environmental samples (27). For example, using techniques such as
172 single-cell genome amplification (38) or shotgun metagenomics (39) it is now possible to reconstruct the entire genomes of representative taxa directly from the environment without
174 cultivation. As the availability of (near-complete) genome sequences continues to increase, we may eventually be able to revert to single marker genes as reliable predictors of genotypic traits
176 (40).

Nevertheless, it is still a challenge to link these genotypic traits with the phenotypic traits
178 on which natural selection is acting. One promising approach for identifying the genotypic traits that underpin ecological differentiation, and thus the phenotypic traits that affect fitness,
180 combines genomic and transcriptomic/proteomic analyses of closely related populations sampled in their natural environments to detect signatures of directional selection (41). These signatures
182 refer to evidence of positive selection, expression of population-specific genes, and differential expression of shared genes when two populations co-occur in the same environment. Initial

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applications of such approaches have confirmed laboratory-based findings regarding the important role that the evolution of gene expression has in the early stages of ecological differentiation (41). Extending these approaches to time series analyses of either laboratory isolates or *in situ* populations may help elucidate the microevolutionary underpinnings of fitness differences for microorganisms under different environmental conditions.

D. Evidence of rapid evolution in microbes: from the lab and into the wild

1) Experimental evolution – Not long after the publication of *On the Origin of the Species*, scientists began to design evolution experiments with microorganisms. For example, in the 1870s, William Dallinger conducted selection experiments where he challenged protozoa to increasing temperatures (42) and by the middle of the 20th century scientists were conducting studies that explored the rapid evolution of virus-resistance by bacteria (43, 44). Since then, methods and approaches used to study the evolutionary ecology of microbial populations have been refined. Arguably, a new era of experimental evolution was initiated by Richard Lenski and colleagues in the late 1980s. One of the ongoing long-term experiments involves the semi-continuous culturing of replicate (n = 12) *Escherichia coli* populations. Conceptually, the experiment is fairly straightforward: 1% of cells from a culture are transferred into fresh medium (glucose-supplemented minimal broth) on a daily basis. A critical feature of most experimental evolution trials is the ability to keep populations from different time points in suspended animation. This is typically achieved by storing cells (either single colony isolates or mixed populations) in a cryoprotectant (e.g., glycerol or DMSO) at -80 °C. The cyropreserved cells can then be resurrected and used to make comparisons among ancestral and derived lineages. For example, one might examine how traits such as cell size, colony morphology, or the ability to use different substrates changes over time (45, 46). Scientists can also use this "fossil record" of

cryopreserved isolates to ask questions about how historical contingencies set the stage for the evolution of novel traits (47). Moreover, experimental evolution trials allow one to identify the genetic basis for neutral and adaptive evolutionary change. For example, it is now possible to resequence whole-genomes of derived isolates from a long-term experiment and identify mutations that arise compared to an ancestral reference strain (48) (Fig. 2). This approach can help reveal whether phenotypic changes are controlled by mutations in structural genes or regulatory genes (the latter is often found to be true). Transcriptomics is another tool that is providing new insight into how populations phenotypically evolve, for example along environmental gradients (49). Collectively, experimental evolution trials allow one to estimate rates of neutral and adaptive evolution within an experimental unit. Furthermore, because experimental evolution trials are fairly easy to replicate, one can also assess the degree to which strains diverge, converge, or evolve in parallel across experimental units (50).

It is common in experimental evolution studies to quantify the relative fitness of a derived population to the ancestral population. This is typically achieved by conducting "head-to-head" experiments where two populations are mixed and allowed to compete for some given amount of time. Growth rates can be estimated as $[\ln(N_{t_f})/\ln(N_{t_0})]/t$ where $\ln(N_{t_f})$ is the natural logarithm of cell densities of a given population at the end of a competition experiment that runs for time t and $\ln(N_{t_0})$ is the natural logarithm of cell densities of a given population at the beginning of the experiment. From this, relative fitness can be estimated as the ratio of growth rates for the derived and ancestral population, respectively. However, when two strains are mixed, it can be difficult to differentiate the competing cell lines. This complication to estimating relative fitness can be overcome through the use of a marker gene that provides a means to select or distinguish different populations. For example, one could select for neutral markers, such as

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lactose utilization, and then enumerate via plating with and without lactose (51). Other strategies might involve insertion of green fluorescent protein or selection for antibiotic resistance (52), but researchers must be aware of how the associated fitness costs of a marker could potentially confound inferences that would be made about evolutionary trajectories. Another strategy is to compete ancestral and derived cell lines against a third party "tester" strain (53, 54), but scientists must be comfortable with the assumption that the tester strain interacts with the ancestral and derived strains in ecologically similar ways.

Over time, the traditional approach to experimental evolution with *E. coli* (55) has expanded to accommodate different taxa, environmental conditions, and species interactions. In addition to batch cultures, microbiologists can set up experimental evolution trials using continuous cultures, or chemostats. One benefit of using chemostats is that there is a constant inflow of fresh media, which means that microbes do not experience fluctuations in physiological conditions that are typical of a batch-culture environment. Second, by altering medium composition or environmental conditions, researchers have the ability to closely control the growth-limiting factor (e.g., nitrogen, phosphorus, light) of a population in a chemostat. Third, mathematical theory has been developed and applied to microbes in the chemostat environment (56), which allows researchers to identify key parameters such as resource uptake or predation defense that are under selection (57). Although chemostats are ideal for studying evolution of planktonic microorganisms, continuous culture techniques have also been developed for studying biofilm-forming strains (58). Other creative variations have been used to study evolution in environments that deviate from the assumptions of spatial homogeneity in the chemostats. For example, through the use of liquid handling robotics on 96-well plates,

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researchers have been able to simulate eco-evolutionary dynamics that occur when species move among patches in heterogeneous landscapes (59).

2) Evolutionary ecology in the wild – Laboratory-based studies have contributed immensely to our basic understanding of microbial evolutionary ecology. However, it is not clear whether the processes contributing to, for example, the rise to dominance of specific genetic variants, are similar under laboratory and natural conditions. For example, a recent study demonstrated that the adaptive diversification of *Pseudomonas fluorescens* was greatly reduced via interactions with a diverse soil microbial community (60). These types of evolutionary dynamics are highly dependent on the population-genetic environment (e.g., the importance of genetic drift owing to effective population size) along with other chemical, physical, and biological processes, which is almost certainly more variable and less controllable in nature than in the laboratory.

In particular, gene flow represents major challenge when studying evolutionary ecology of free-living microorganisms in nature. Immigration may be less of a concern when studying relatively "closed" environments, such as acid mine drainage (AMD) ecosystems, which are biogeographically isolated from other source populations that are adapted to such unique conditions (e.g., low pH and high metal concentrations). After reconstructing the genome of a bacterial population in one of these AMD sites, researchers were able to track the accumulation of fixed single nucleotide polymorphisms (SNPs) over time (Fig. 3). From this, they were then able to estimate an evolutionary rate of 1.3×10^{-9} substitutions per nucleotide per generation, which is similar to rates reported in many laboratory experiments (61). Using the AMD as a model system, researchers were then able to reconstruct the timeline of recent divergence events and demonstrate the rise of dominance for mutations in different lineages resulting from positive

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276 selection and drift. Similar patterns of periods of positive selection alternating with periods of drift were observed in a study tracking the evolution of *Pseudomonas aeruginosa* in the lungs of 278 cystic fibrosis patients (62).

When studying evolution in the wild, just as in laboratory studies, researchers need to 280 determine the relative importance of genetic drift and positive selection on the rise in dominance of particular variants. This can be accomplished by calculating dN/dS ratios for the genes 282 affected by mutations (63). The dN/dS ratio, which can be applied to specific loci or entire genomes, calculates the number of non-synonymous mutations across all available non- 284 synonymous sites relative to the number of synonymous mutations across all available synonymous sites. It is becoming more common to estimate dN/dS ratios using metagenomic 286 data from environmental samples (64, 65). Care has to be taken, however, when interpreting the dN/dS ratio for a population because the metric makes assumptions that are only valid for 288 comparisons between more distantly related organisms (66). Methods are available to correctly assess the directionality (positive, negative, neutral) of selection (67, 68), but microbiologists 290 still must be aware that high error rates associated with different sequencing technologies will be misinterpreted as mutations (69). Nevertheless, the dN/dS ratio can provide clear insight into the 292 relative importance of evolutionary processes in some instances. For example, tight population bottlenecks between insect generations resulted in strong effects of genetic drift on a bacterial 294 endosymbiont (*Buchnera*), which resulted in rapid reductive genome evolution (70).

It is well established from the study of microbial isolates that homologous recombination 296 and lateral gene transfer are important processes that influence microbial divergence (20, 22, 71). Through the use of environmental genomics (metagenomics), it has been shown that these 298 evolutionary processes are also important for the generation of population-level diversity. In

particular, metagenomic studies are starting to answer outstanding questions regarding the relative importance of recombination and mutation (21, 72, 73). To fully document the nature and rate of introduction of new genes into genomes over time, it is critical to reconstruct (near-) complete population genomic datasets for each time point. Two recent time-series analyses of the gut colonization of preterm infants show the potential of metagenomics to track the varying gene content of closely related microorganims and relate it to the varying abundances of these strains over time (74, 75). Comprehensively tracking the flow of genes in and out of populations remains an unmet challenge however, which may be aided by emerging longer-read DNA sequencing technology.

E. Spatial scale and the evolutionary ecology of microbes – The example of the AMD system (Fig. 3) is unique because we can assume that the immigration and establishment of novel genotypes from similar ecosystems is rare. In more "open" natural systems, spatial processes are critical for understanding the evolutionary ecology of microorganisms. The movement of individuals and the resulting gene flow between sub-populations can have strong effects on allele frequencies and the evolutionary trajectory of the local and meta-*population* (i.e., the collection of geographically separated, but interacting populations of a species). Specifically, reductions in gene flow increase divergence between isolated populations, which in turn can lead to speciation via selection or drift. Migration (or dispersal) is also an important ecological process that can influence the assembly of *communities* (76). For example, dispersal limitation may contribute to high levels of β-diversity (i.e., high compositional turnover among sites), while high rates of dispersal can create "mass effects" that allow for the persistence of competitively inferior species in a local community (77).

Owing to their small size, it is assumed that microbes can be carried long distances via passive mechanisms or through close association with larger host organisms. Through the use of analog microspheres, it has been shown that microbial-sized particles can be transported up to 2 km within days depending on weather conditions (78). In other studies, it is estimated that a bacterial cell in the atmosphere has a residence time of 2-15 days (79), which in some cases can lead to the continental-scale dispersion of microorganisms (80). These high dispersal rates could result in the cosmopolitan distribution of microbial populations. However, multiple lines of evidence suggest that this is not entirely the case. Using multilocus sequencing of hyperthermophilic archaea, it was shown that a S*ulfolobus* sp. had high F_{ST} values (a population genetic index that quantifies the variance in allele frequencies between populations) consistent with the view that there was minimal mixing among hot spring environments spanning a 6000 km sample gradient (81). Pairwise genetic divergences estimated from S*ulfolobus* isolates were positively correlated with geographic distance providing further evidence that not all microorganisms have panmictic distributions (Fig. 4).

A classic way to examine the spatial patterns of biodiversity for entire communities is through the construction of species-area relationships (SAR). These relationships describe diversity with the power function: $S = cA^z$, where S is species richness, A is area, and c and z are constants. When S and A are plotted on a log–log scale, the slope, z, can be used to quantify the rate at which new species are encountered with increasing sampling area. When estimated for microbes, z-values tend to be much lower than macroscopic organisms (e.g., plants and animals), but significantly greater than zero (82). It has been hypothesized that these patterns arise from dispersal limitation, but could also be attributed to other factors including the fact that environmental heterogeneity tends to scale positively with geographic distance (82). In a recent

meta-analysis, geographic distance was found to have a significant effect on microbial composition in half of the studies. Approximately 10% of the observed variance could be uniquely attributed to geographic distance while ~25% was uniquely attributed to measured environmental factors, and ~15% to combined effects (83).

If microorganisms experience dispersal limitation in patchy environments, then we should expect to find evidence for local adaptation in at least some microbial populations. Local adaptation occurs when the performance or fitness of an individual is higher in its "home" vs. "away" environment. Evidence for local adaptation is often obtained from transplant studies and suggests that the strength of selection caused by local conditions exceeds the strength of gene flow. To test for local adaptation, heterotrophic soil bacteria were isolated from multiple sites within a one hectare old-growth forest and cultured in soil medium derived from local and distant sites (84). When the authors focused on fast growing isolates, they found that bacteria had the highest fitness on locally derived medium and fitness decayed exponentially on media derived from more distant sites (Fig. 5). Such findings led to the conclusion that edaphic heterogeneity and limited dispersal, relative to evolutionary rates, created complex fitness landscapes for bacteria at relatively small spatial scales. Microorganisms may also show signs of local adaptation to the types of organisms they interact with. For example, many bacteria have to contend with the selective pressures caused by predation and parasitism. It is known that many bacterial populations can evolve resistance to phage, but less is understood about how this evolutionary adaptation plays out over larger spatial scales. Such questions form the basis of the Geographic Mosaic Theory of Coevolution (85), which has been addressed using laboratory experiments (86), but also natural communities. For example, bacteria and phage were isolated from 25-cm x 25cm grids for two soil samples that were separated by 100 m (87). The bacterial

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368 isolates were then challenged with co-occurring and geographically distant phage populations. On average, phage were 9% more infective on their local bacterial hosts. Phage fitness
370 diminished when challenged with bacteria that were only centimeters away, suggesting that viruses may be ahead of bacteria in the co-evolutionary arms race and that biotic eco-
372 evolutionary interactions are not always swamped out by rampant dispersal.

374 **F. Temporal scale and the evolutionary ecology of microbes** – We have pointed out in this chapter that microorganisms attain large population sizes, can have short doubling times, and in
376 some cases can exchange genes with distantly related taxa. Combined, these characteristics set the stage for evolution to occur on ecologically relevant or "rapid" time scales. Perhaps the best
378 evidence of this comes from the study of *E. coli* in batch culture. After being inoculated into fresh medium, *E. coli* enters exponential growth phase within just hours. During this time, cells
380 grow at their maximum potential and rapidly deplete resources. As a result, per capita growth rates decline and *E. coli* enters a stationary phase, which is followed shortly thereafter (2- 5 days)
382 by a death phase where population densities decline by about an order of magnitude. Intriguingly, cell densities can remain fairly constant after the death phase for extended periods
384 of time (years), due in part to a phenomenon referred to as growth advantage in stationary phase or "GASP" (Fig. 6) (88). Although the aggregate population appears relatively stable, bacteria
386 are extremely dynamic during periods of prolonged starvation. Ecologically, this can be attributed to the fact that some individuals die and release their cellular constituents back into the
388 environment, while other individuals assimilate this material along with other metabolic byproducts for growth and reproduction. Evolutionarily, it has been shown that cannibalistic
390 subpopulations are variants that arise and invade the system in a negative frequency dependent

manner (89). GASP-related research has led to the prevailing view that starvation is not only a strong selective agent, but it also alters the rates of *de novo* mutation either through methyl-directed mismatch repair (MMR) or the SOS response, which activates error-prone polymerases (e.g., PolIV and PolV) (88, 90). The GASP phenomenon demonstrates that starvation stress is a proximal cue that that leads to the accumulation of beneficial mutation (88), while also providing an explanation for the persistence of the population under resource-limited conditions (Fig. 6).

Microorganisms can also contend with unfavorable conditions (including starvation) by hedging their bets and entering a reversible state of reduced metabolic activity, or dormancy (Fig. 7). Dormancy has evolved many different times in the tree of life and is a functional trait that allows genotypes or even entire populations to avoid going extinct. For example, viable microorganisms have been retrieved from ancient materials (e.g., permafrost and amber) that, in some cases, are hundreds of millions of years old (91). The resurrection of populations from so-called "seed banks" has obvious evolutionary implications, but is also important for maintenance of biodiversity and the functioning of communities (92). There are a variety of ways to estimate dormancy in microbial communities. Some taxa produce spores, cysts, or akinetes when they enter inactive state, but these morphological traits are not reliable indicators for dormancy for all microorganisms. Single-cell assays based on fluorescent in situ hybridization or the uptake of tetrazolium stains can be useful for estimating the activity of microbial cells (93). Recently, inferences about the metabolic activity of bacteria have been made by examining the 16S region of ribosomal RNA genes (rDNA) and ribosomal RNA (rRNA) (94). Justification for this approach is as follows: in general, rDNA is a stable molecule that is widely used to infer the presence (and *potential* activity) of a population. In contrast, rRNA is an ephemeral molecule that is only produced by growing cells, which require ribosomes for protein synthesis (95). As

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such, rRNA has been used for identifying active taxa in complex microbial communities (e.g., (96). Although RNA:DNA is strongly correlated with microbial growth rates in laboratory settings (97), concerns have been raised about applying this technique to broad ranges of taxa (98). An alternate approach is to focus on genes (e.g. toxin-antitoxin modules or resuscitation promoting factors) that are directly involved in the transitions between active and dormant metabolic states (92).

Last, epigenetic processes can also affect the temporal scale of eco-evolutionary processes by allowing organisms to rapidly respond to environmental signals and pass this response on to its offspring (99). Epigenetic processes refer to non-genetic mechanisms (i.e., not directly related to differences in nucleotide sequence) that cause variability in gene expression that can result in phenotypic variation subject to natural selection. While a variety of systems are referred to as epigenetic, best studied is the system based on DNA methylation and interactions with histone proteins. Histone-DNA interactions condense DNA and render these stretches of the DNA unavailable for transcription, thus effectively shutting down gene expression. Epigenetic marks (methylations) are accrued during an organism's life in response to environmental or developmental cues, are reversible, and, importantly, they are heritable. Although epigenetic studies have mostly focused on eukaryotes, the mechanism is relevant in bacteria as well (100, 101) and genome-wide determination of methylation patterns can readily be performed using via sequencing approaches (102). While this area is relatively new, and the implications on evolutionary and ecological processes are still unclear, there is evidence that phenotypic variation between bacterial subpopulations of the same species can be caused by heritable variability in DNA methylation patterns. Methylation plays an important role as a signal for a variety of bacterial cellular processes; for example, repair enzymes use them to differentiate the

original (methylated) template DNA strand vs. the newly (temporarily unmethylated) copied strand during replication. Maintaining stretches of DNA in the hemi- or unmethylated state beyond the replication phase can affect gene expression and has been shown to be the mechanism for several phase-variable phenotypes, including the expression of pili in uropathogenic *E. coli* (101). Epigenetically controlled phase variation-based creation of subpopulations can be seen as another example of bet hedging. The ability to transmit a fitness-affecting phenotype acquired through epigenetic modifications can influence the evolution of a lineage in multiple ways, and is another mechanism to keep in mind when determining the impacts of eco-evolutionary dynamics on microbial systems.

G. Eco-evolutionary feedbacks in microbial systems – We have emphasized that microbial communities are taxonomically, phylogenetically, and metabolically diverse. We have also shown that microorganisms have the capacity to evolve on ecologically relevant time scales. Together, these features set the stage for eco-evolutionary feedbacks. From the ecological side of the feedback, it is well established that species interactions (e.g., competition, parasitism, or mutualisms) can affect evolutionary processes such as adaptation and speciation (Fig. 1). From the evolutionary side of the feedback, evolutionary changes (e.g., selection for traits) can modify population dynamics, species interactions, and even ecosystem processes (103). Over the past decade, evidence has been accumulating that eco-evolutionary feedbacks are important for understanding plant and animal dynamics (3, 104, 105). In the last section of this chapter, we highlight examples where eco-evolutionary feedbacks are important for understanding how microbes interact with each other and their hosts.

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1) Feedbacks involving antagonistic interactions – Antagonistic interactions between predators and prey or hosts and parasites can often give rise to eco-evolutionary feedbacks. In microbial systems, clear evidence of this can be found when studying bacteria-phage dynamics. Lytic phage can reduce the population size of sensitive bacteria by orders of magnitude within a short period of time. This strong top-down force creates strong selective pressure for phage resistance. Bacteria have evolved various ways of resisting phage attack including the modification of surface receptors, DNA restriction-modification systems, and clustered regularly interspaced short palindromic repeat (CRISPR) immunity (106). It is generally assumed that the benefits afforded by the specialization of phage resistance come at a cost (54, 107). For example, the loss or configuration change of a receptor molecule that interferes with phage attachment to the cell surface can also reduce rates of resource uptake (108).

The fitness costs associated with predator defense traits are critical for understanding microbial population dynamics involving eco-evolutionary feedbacks. For example, the cost of resistance establishes a trade-off between phage defense and resource competition that allows for coexistence of bacterial variants and the ancestral phage population. Both models and empirical evidence indicate that microbial population dynamics are highly sensitive to this type of tradeoff. In a chemostat study of a eukaryotic alga (*Chlorella*) and a predatory rotifer (*Brachionus*), it was shown that periodic selection on resource acquisition and predator defense led to surprising population dynamics (109). Specifically, the authors anticipated relatively fast cycles where peaks in predator abundances tracked peaks in prey abundances by one-quarter of cycle as predicted by general ecological theory. Instead, they found that the cycles were much slower. Moreover, the predator and prey densities were almost exactly out of phase (109). Subsequently, it was put forth that trophic interactions can be masked by rapid evolution caused by antagonistic

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482 species interactions giving rise to "cryptic" population dynamics (6). These types of controlled studies may help explain the absence of classic predator-prey cycles between bacteria and phage 484 in natural systems when analyzing data at a coarse phylogenetic resolution (110).

Rapid evolution caused by antagonistic species interactions can also affect ecosystem 486 processes. Phage are highly abundant in the open ocean, and can account for a substantial fraction of bacterial mortality. Indirectly, phage are thought to increase the concentration of 488 carbon and nutrient in the oceans by reducing microbial population sizes. In addition, phage lysis events are directly responsible for releasing labile resources into the environment, which can 490 affect global biogeochemical cycles through a process known as the viral shunt (111). How might rapid evolutionary change affect the viral shunt? This question was explored in a 492 chemostat experiment with *Synechococcus*, a marine picocyanobacterium, and an infectious phage (112). *Synechococcus* population densities plummeted after the initial phage attack, which 494 led to significant increases in phosphorus and alterations of the elemental stoichiometry of microbial biomass. However, these effects of phage on nutrient cycling diminished with time 496 owing to the evolution of phage-resistant bacteria. These laboratory results with environmental isolates suggest that rapid evolution may be important when attempting to understand and model 498 the impacts of viruses on microbial food webs.

500 2) Feedbacks involving mutualistic interactions – Although historically overlooked, mutualistic interactions can be important drivers of eco-evolutionary dynamics. Many microbial 502 taxa engage in mutualistic interactions, either with other microbes, or with plants and animals. These mutualisms range from relatively loose associations to obligate endosymbioses. In the 504 case of endosymbionts and their hosts, co-evolution and co-differentiation (parallel evolutionary

paths of symbionts and hosts) have been occurring for millions of years (113). But how dynamic are these interactions on ecological time-scales?

Growing evidence suggests that many microbial-based mutualisms have the potential to rapidly evolve. For example, experimental evolution trials were conducted with a sulfate reducing bacterium (*Desufovibrio vulgaris*) and a methanogenic archaeon (*Methanococcus maripaludis*); two isolates that had no known history of interaction (114). While both populations could be grown in pure culture, the authors attempted to establish an obligate syntrophic mutualism by growing the strains in lactate medium in replicate (n = 24) co-culture. Initially, growth of the co-cultures was unstable, leading to the extinction of one of the populations. In only 300 generations, the evolved co-cultures grew up to 80% faster and produced 30% more biomass, which was the result of evolution by both partners. The stability of this novel mutualism, however, was challenged by mutations that gave rise to more antagonistic variants. In addition, the stability of the mutualism was influenced by the heterogeneity of the environment. Specifically, contributions of the methanogenic partner to the performance of the community were greater in heterogeneous environments (shaken flaks) than homogenous environments (non-shaken flasks), presumably due to the increased exchange of substrates among mutualists. This study uniquely demonstrates the power of controlled experiments to investigate how metabolism, habitat features, and behaviors such as cheating might influence the development and stability of microbial mutualisms.

Microorganisms can also readily establish mutualistic relationships with plant and animal populations. This has become an important topic of research given concerns about the accelerating rate of global change. Some species may be able to persist in novel or changing environment through ecological strategies such as phenotypic plasticity, behavioral

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modifications, or migration to more favorable habitats. A second strategy is for the plant or animal population to adapt to new conditions, but it remains unclear whether macroscopic organisms have the capacity to evolve at a fast enough pace to keep up with environmental change (115). A third strategy is for plant and animal populations to "outsource" adaptive traits to symbiotic microorganisms. This concept has been articulated in the Hologenome Theory of Evolution (116). The major tenants of this theory are that i) all plants and animals establish symbiotic relationships with microbes, ii) symbiotic microorganisms can be vertically transmitted via different mechanisms, iii) microbe-host interactions affect the fitness of the "holobiont", and iv) genetic variation of the holobiont can be enhanced through the rapid recruitment of microorganisms from diverse communities. The Hologenome Theory of Evolution was initially developed to help explain a coral-bleaching phenomenon. Specifically, because corals lack adaptive immunity, it was hypothesized that they could recruit beneficial microorganisms from the marine environment to prevent infection from pathogenic bacteria (117). Since then, some of these ideas have been tested in other systems as well. For example, when challenged by drought stress for multiple generations, reciprocal transplant experiments revealed that plant fitness was strongly affected by the rapid shifts in soil microbial communities (118). The Hologenome Theory of Evolution may also have important implications for understanding macroevolutionary processes. For example, within just a few generations, diet-driven shifts in the composition of commensal bacteria altered the mating preferences of *Drosophila melanogaster*, which could lead to prezygotic reproductive isolation (119). In addition, it was recently shown that hybrid lethality among closely related wasp species (*Nasonia* sp.) was due to negative epistasis (i.e., mismatched gene-gene interactions) between the host genome and the gut microbiome (120).

552 **H. Conclusion.** Over the past 50 years, biologists' views regarding the interplay between ecology and evolutionary biology have dramatically changed (105). For example, Dobzhansky 554 famously stated that "Nothing in biology makes sense except in the light of evolution" while Peter and Rosemary Grant retorted with "Nothing in evolutionary biology makes sense except in 556 the light of ecology". More recently, it seems we have arrived at the notion that "Nothing in evolution or ecology makes sense except in the light of the other" (121). We argue that 558 microbiologists are uniquely poised to make advances to the field of evolutionary ecology. In fact, major advances to this area of research have already been made owing in large part to the 560 amenability of microbial systems to laboratory-based study. While it is conceivable that all of these findings are relevant to *in situ* conditions, laboratory experiments deviate from real-world 562 systems in temporal and spatial scale, and in the level of complexity of ecological interactions. In this chapter, we have highlighted (i) that evolutionary rates and processes are similar in the 564 laboratory and in the wild, (ii) that in laboratory settings, ecological and evolutionary processes occur on similar timescales, and both need to be taken into account to explain experimental 566 observations, (iii) what is currently known regarding temporal and spatial processes that may impact *in situ* eco-evolutionary feedbacks, and (iv) some examples where eco-evolutionary 568 feedbacks have been shown to be relevant in the wild.

Similar to plant and animal ecologists and evolutionists, we are only at starting to answer 570 the question of how relevant eco-evolutionary feedbacks are in understanding community structure and functional stability. As summarized in the first section of this chapter, the nature of 572 microbial systems may give us the chance to acquire insights much faster, contributing not only

to our own field's progress, but also to the understanding of universal eco-evolutionary principles, applying to all forms of life.

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FIGURE CAPTIONS

882

Fig. 1. Conceptual diagram depicting feedbacks between ecological and evolutionary processes. 884 Within the domain of ecological processes, there are interacting hierarchical levels of organization (individuals, populations, communities, and ecosystems), which can affect 886 microevolutionary processes (i.e., anagenesis) and macroevolutionary processes (cladogenesis). Reciprocally, evolutionary processes can affect ecological processes. The strength of these 888 feedbacks is influenced by the time scale at which ecological and evolutionary processes take place, but also by factors such as mutation rates, genetic drift, gene flow/disperal, and the 890 diversity of a biological community. Adapted from (8)

892 **Fig. 2.** Relationship between phenotypic and genotypic change over time. Data originate from competing and evaluating fitness differences between ancestral and evolved *E. coli* lineages. 894 While fitness increases saturate over time, fixed genetic changes continue to increase linearly over time. This pattern highlights some of the difficulties when trying to translate genotypic 896 traits to phenotypic traits. Adapted from (48).

898 **Fig. 3.** Determining rates of evolutionary in the wild. (A) Samples were collected from one location in the AMD system (C75) and *de novo* sequence assembly of sequencing reads led to 900 the reconstruction of a genome for the dominant *Leptospirillum* Group II at the site (Type III). (B) Read recruitment of all 13 sequence datasets generated from C75 samples over five years to 902 the Type III reference genome allowed for the identification of additional fixed mutations and estimation of the nucleotide substitution rate. Lower frequency mutations could be observed in

each of the datasets as well, but only fixed variants are included for rate calculations. Adapted from (61)

Fig. 4. Pairwise sequence divergence of *Sulfolobus* populations isolated from a global survey of hot-spring ecosystems scales positively with geographic distance providing evidence against the view of panmicitic microbial distributions. Adapted from (81)

Fig. 5. Evidence for local adaptation demonstrating the distance decay for the relative fitness of soil bacteria grown on resources from different geographic locations. Adapted from (84)

Fig. 6. Some bacteria can rapidly evolve in response to starvation. The upper panel shows a typical growth curve of *Escherichia coli*. When populations deplete resources they enter stationary phase followed by a death phase. Subsequently, *E. coli* (and other types of bacteria) can enter growth advantage in stationary phase (GASP), where novel starvation-resistant mutants evolve and invade a system as depicted by the colored curves in the top panel (adapted from 88) and the conceptual model in the lower panel.

Fig 7. When challenged with conditions that are suboptimal for growth and reproduction, some microorganisms can enter a reversible state of reduced metabolic activity, or dormancy. The size of the active population is determined by the net reproductive rates, losses due to mortality, and losses due to dormancy. The size of the dormant population is determined by the rate at which active individuals transition into dormancy, the mortality rate during dormancy, and resuscitation

926 from dormancy. This bet-hedging strategy is important for the maintenance of microbial biodiversity. Adapted from (94).

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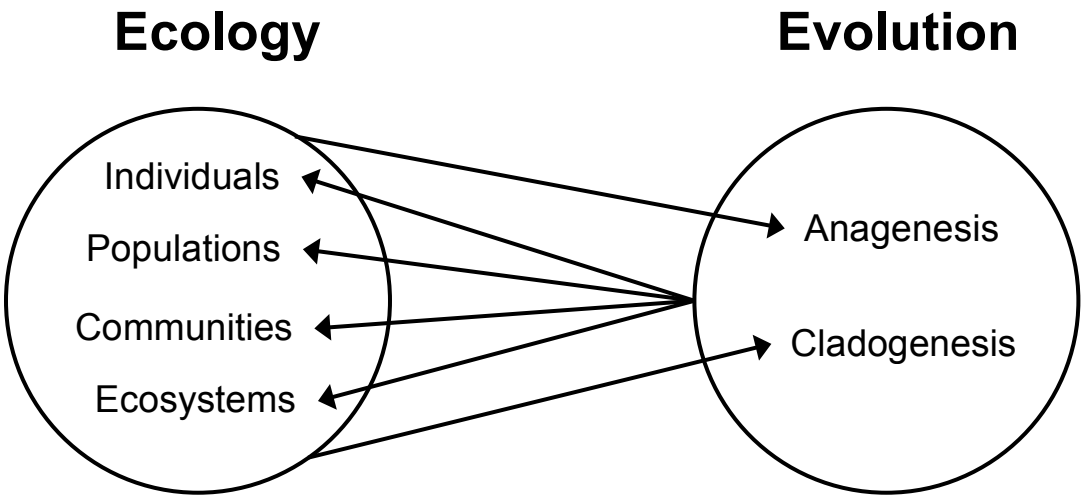
Fig. 1

934



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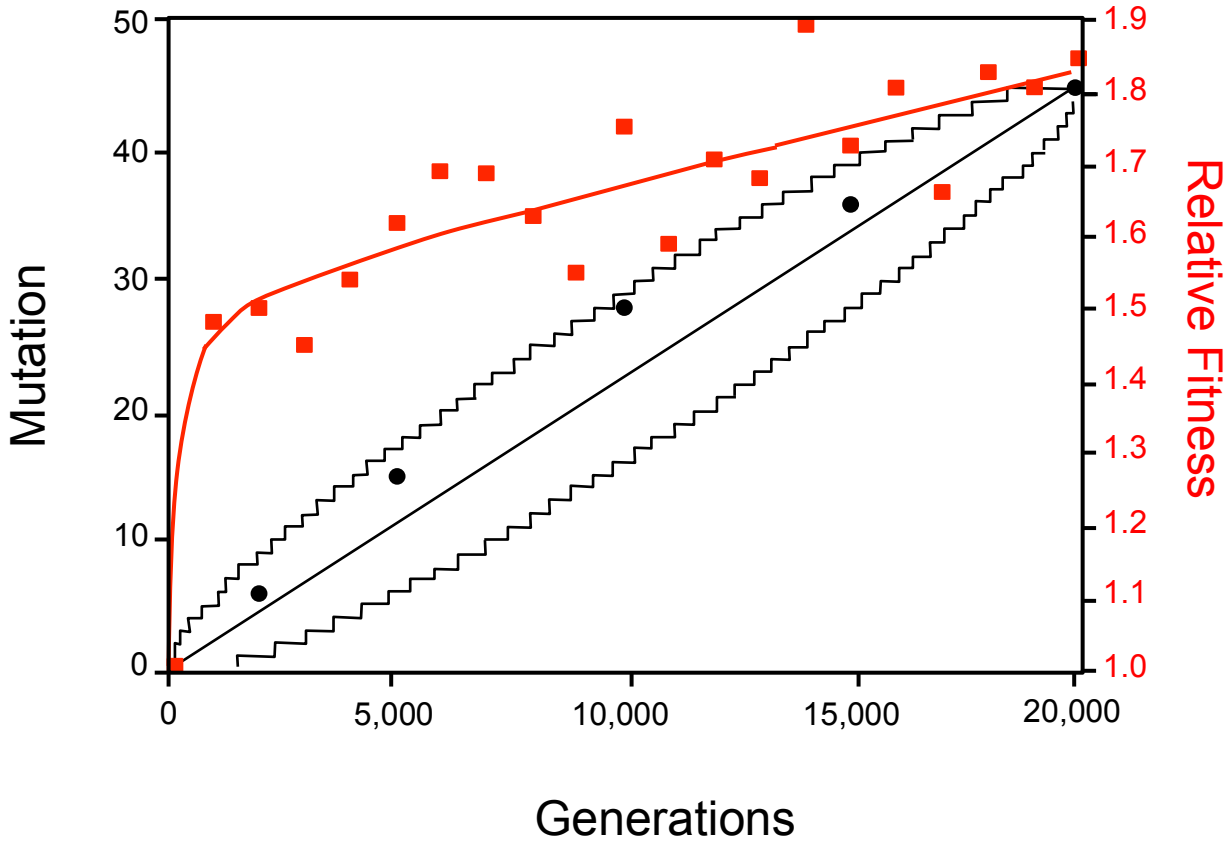
936 Fig. 2



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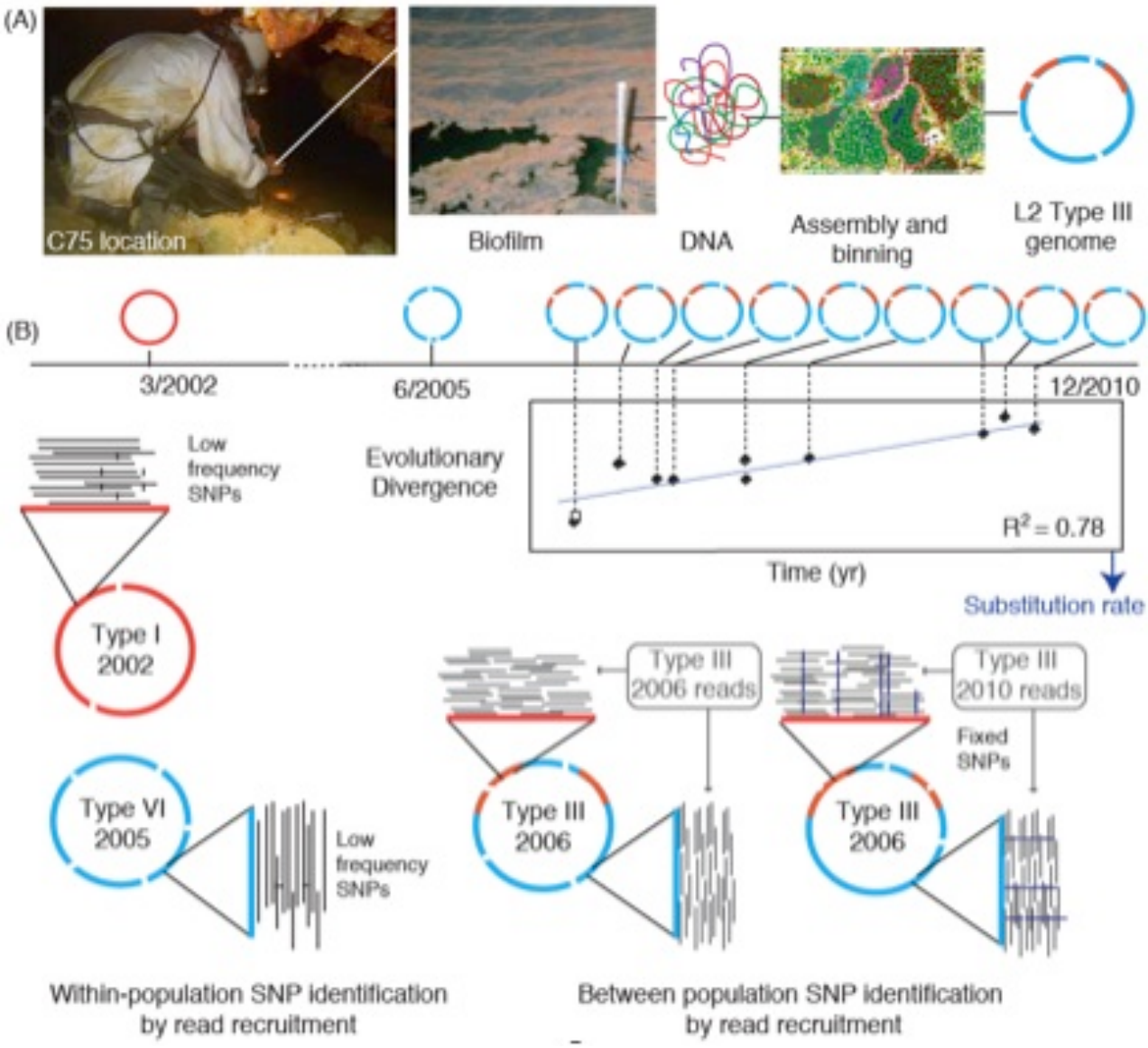
938 Fig. 3



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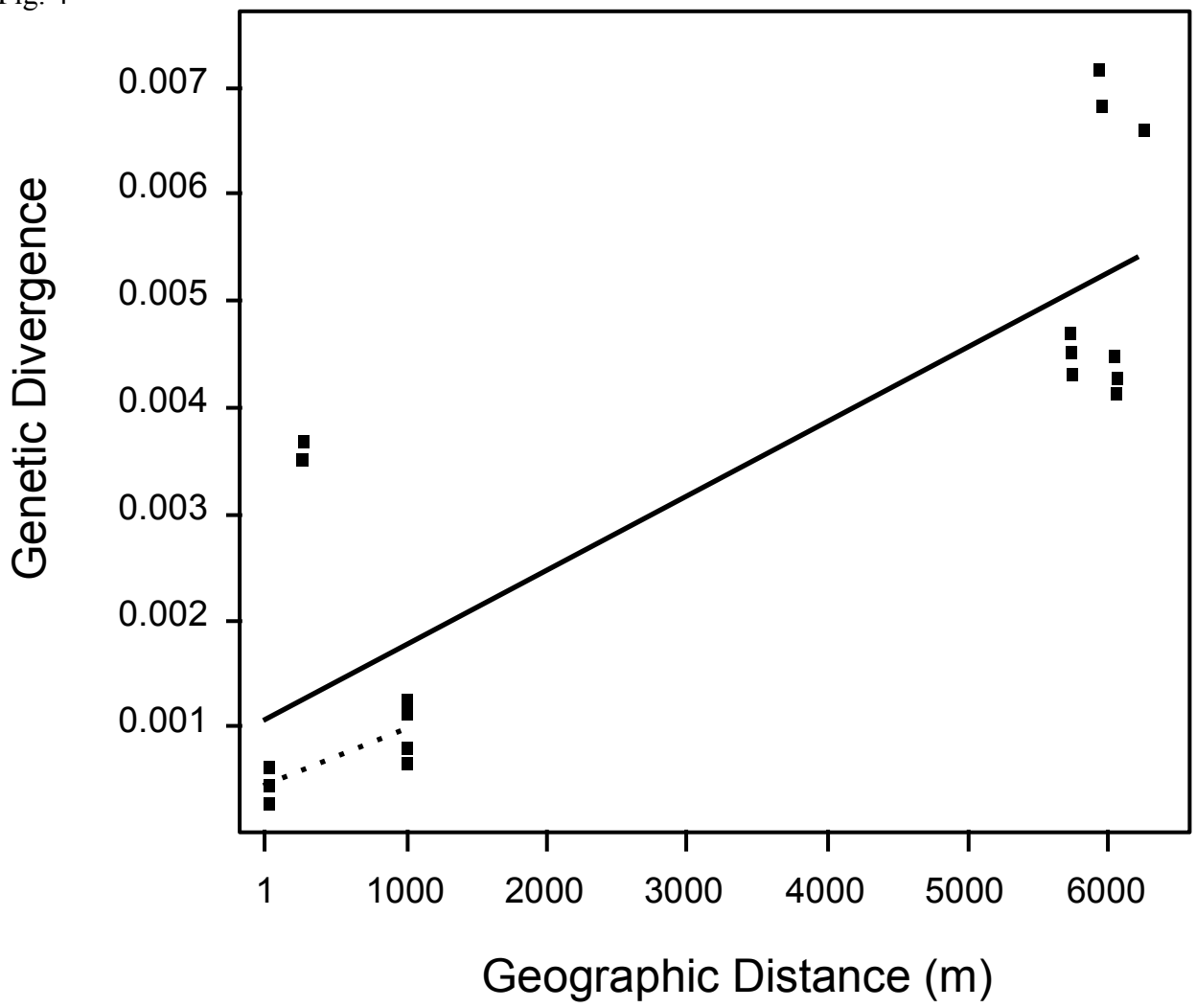
940 Fig. 4



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942 Fig. 5

944

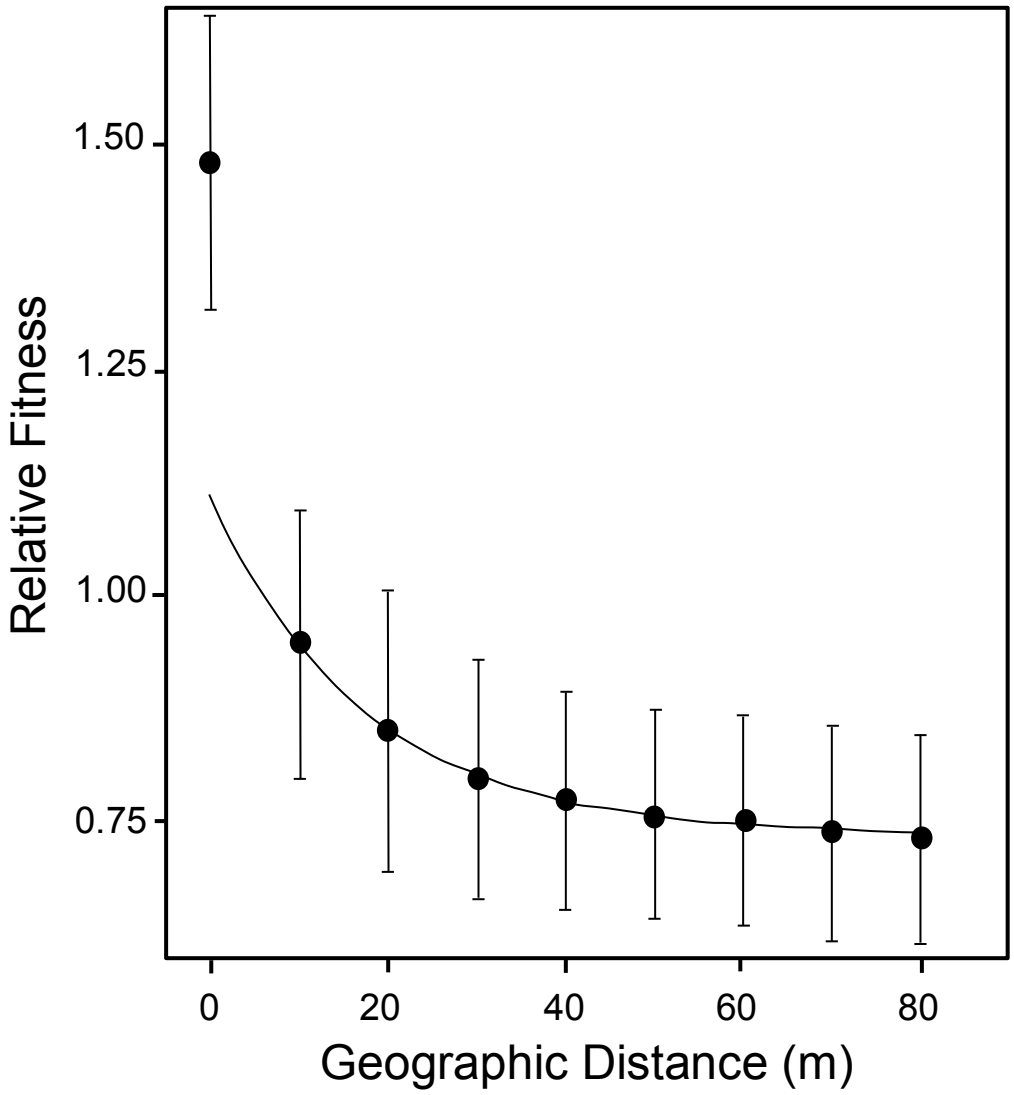

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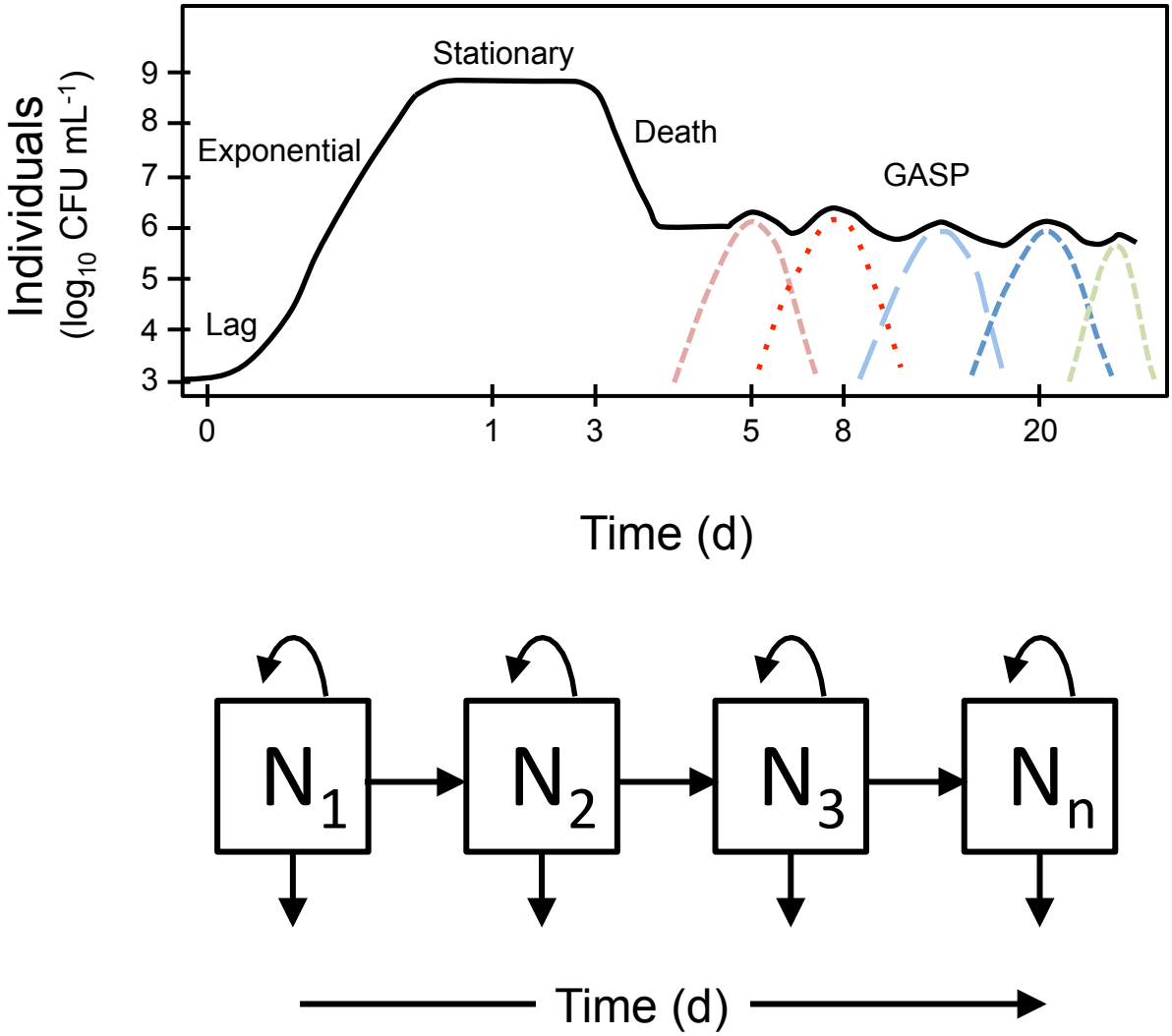
Fig. 6

946



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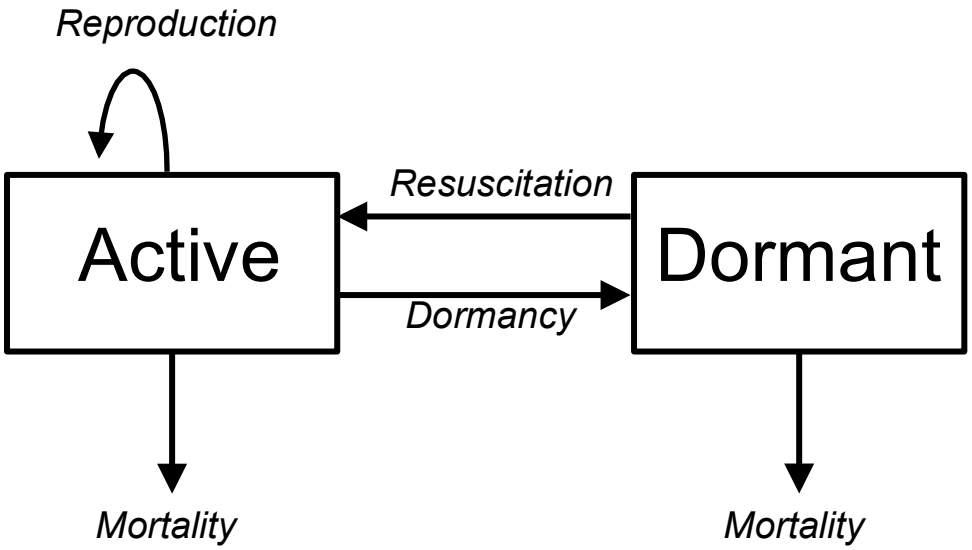
948 Fig. 7



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