Evolution with a seed bank: the population genetic consequences of microbial dormancy

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ABSTRACT

Dormancy is a common bet-hedging strategy that allows organisms to persist through biotic and abiotic conditions that are sub-optimal for growth and reproduction by reducing their metabolic activity. The ability to enter and exit a dormant state allows a population to maintain a reservoir of genetic and phenotypic diversity that can contribute to the long-term survival of the population (i.e., a seed bank), a potentially adaptive strategy has long been of interest to ecologists and evolutionary biologists. However, we know comparatively little about how dormancy influences the fundamental evolutionary forces of genetic drift, mutation, selection, recombination, and gene flow. Here, we discuss how seed banks affect the fundamental forces that underpin evolution by reviewing existing population genetic models, implementing novel simulations, and determining how and when dormancy can influence evolution as a population genetic process. Our simulations show that dormancy can only influence selection under certain demographic conditions, suggesting that the active portion of a population can continue to undergo adaptive evolution even in the presence of a large seed bank. We then extend our analysis to examine how seed banks can alter macroevolutionary dynamics and determine whether metabolically inactive microbial populations are engaging in dormancy as a life history strategy. We propose that by examining the population genetic and macroevolutionary implications of dormancy as a life history strategy we can understand the extant that seed banks influence microbial evolutionary dynamics.

KEYWORDS: microorganisms, evolution, population genetics, macroevolution, seed banks, dormancy, bacteria, bet-hedging

INTRODUCTION

Nature is rarely predictable. Resource availability, disease pressure, and temperature are just a few of the abiotic and biotic factors that fluctuate over space and time. Variation in these, and other, biotic and abiotic factors have important consequences for the growth, survival, and reproduction of individuals. Many taxa respond to variability in their environment by entering a reversible state of reduced metabolic activity, a phenomenon known as dormancy (Lennon & Jones, 2011). Dormancy is an adaptive trait that has independently evolved multiple times across the tree of life (Guppy & Withers, 1999). By entering a dormant state individuals can endure conditions that are sub-optimal for growth and reproduction, which results in the net increase of a population's long-term geometric fitness (Cohen, 1966). However, dormancy comes at a cost. This cost not only reflects the missed opportunities for reproduction, but also the energetic investment required to produce resting structures (Bradshaw et al., 1998; Cáceres & Tessier, 2004; van Bodegom, 2007). Despite these costs, dormant individuals accumulate in many systems and form a seed bank (Locey et al., 2017), which serves as a reservoir of genetic and phenotypic diversity (Fig. 1). Seed banks have important implications for a range of ecological phenomena, perhaps most central is the maintenance of biodiversity. Specifically, dormancy reduces interspecific competition allowing for coexistence, a mechanism known as the storage effect (Chesson & Warner, 1981; Chesson, 1994). Seed bank mediated diversity has consequences for other ecological processes including patterns of succession (Marks, 1974; Bazzaz, 1979; Lennon & Jones, 2011), community responses to perturbations (Kalamees & Zobel, 2002), and ecosystem processes (Wang et al., 2014).

Dormancy also has important consequences for evolution. For example, rates of phenotypic evolution are reduced in freshwater zooplankton that persist in a dormant state

70 (Hairston & De Stasio, 1988). Similarly, dormancy has also been found to be associated with a higher rate of lineage diversification in plants (Willis et al., 2014). However, to fully understand
72 how dormancy influences evolution, it is necessary to understand how it affects the relative strength of the fundamental forces that govern the rate and direction that allele frequencies
74 change over multiple generations. Evolution is a population genetic process governed by both deterministic and stochastic forces, the relative strengths of which dictate how genetic diversity
76 is generated, lost, and maintained. Because dormancy as a life history strategy assumes trade-offs that affect fitness, it is subject to the deterministic force of selection if it is sufficiently strong
78 (Cohen, 1966; Templeton & Levin, 1979; Brown & Venable, 1986). In addition, dormancy can trade-off with dispersal as an alternative life history strategy (Venable & Lawlor, 1980; Venable
80 & Brown, 1988; Olivieri, 2001; de Casas et al., 2015), affecting the evolutionary force of gene flow (Vitalis et al., 2004). However, far less progress has been made towards understanding how
82 dormancy influences the stochastic forces of evolution that operate within a single population (e.g., genetic drift, mutation, and recombination). To gain a complete understanding of how
84 dormancy influences evolutionary dynamics it is necessary to consider how it affects the stochastic and deterministic forces that underpin evolutionary biology.

86 While dormancy can influence eco-evolutionary dynamics across the tree of life (Hairston et al., 1999; Willis et al., 2014), it could have a particularly large effect on
88 microorganisms. Dormancy is prevalent across ecosystems, ranging from the human gut to engineered ecosystems to the global ocean (Lennon & Jones, 2011). For example, > 90% of
90 microbial biomass is soils is metabolically inactive (Alvarez et al., 1998; Lennon & Jones, 2011; Blagodatskaya & Kuzyakov, 2013). In addition, microorganisms can alter their metabolically
92 activity in a matter of hours, suggesting that they can quickly transition between active and

dormant states (Fig. 2; Walker & Winslow, 1932; Kaprelyants & Kell, 1993; Votyakova et al.,
94 1994; Ishiguro et al., 2015). Although metabolic transition may occur stochastically (Epstein,
2009), dormancy can also be regulated by environmental cues, such as changes in temperature
96 (Oliver et al., 1995), pH (Keynan et al., 1964), water (Aadnerud et al., 2015), and resource
supply (Dworkin & Shah, 2010). The amount of time that microorganisms spend in a dormant
98 state is variable. Some microorganisms succumb to environmental stress within days (Bale et al.,
1993), while others can persist in a dormant state for prolonged periods of time. For example,
100 viable microorganisms have been retrieved from ancient materials (e.g., permafrost, amber,
halite crystals) that, in some cases, are hundreds of millions of years old (e.g., Johnson et al.,
102 2007). As a consequence, dormant microorganisms can survive for periods of time that far
exceed the average generation time of actively reproducing individuals (Cano & Borucki, 1995;
104 Vreeland et al., 2000). This means that active individuals can continue to evolve while
individuals in the seed bank remains relatively static. Because a considerable fraction of
106 phylogenetically diverse microbial taxa are able to enter a dormant state across disparate
environments, it is likely that dormancy has influenced the evolutionary history of
108 microorganisms. This influence has the potential to extend across evolutionary scales, ranging
from the population genetic processes that underlie the evolutionary dynamics of populations
110 (i.e., genetic drift, mutation, selection, recombination, and gene flow) to the rates that microbial
lineages diverge and go extinct.

112 In this paper, we focus on how dormancy affects microbial evolution. We do this by
synthesizing existing population genetic theory and developing novel simulations that provide
114 insight into how seed banks can affect the fundamental forces that govern the rate and direction
of evolution. We then examine how dormancy influences evolutionary dynamics at longer time

116 scales, including macroevolutionary processes such as speciation and extinction. In addition, we examine environments where organisms are often found in a dormant state to determine whether
118 dormancy is an evolutionarily viable bet-hedging strategy or simply a metabolic response to conditions that are sub-optimal for growth and reproduction (Box 1). Environments do not
120 always improve and if organisms never have the opportunity to reproduce, the ability to enter a dormant state will merely increase the amount of time it takes for a population go extinct.
122 Throughout this paper, we emphasize the importance of using population genetic theory to understand how dormancy as a life history strategy can alter evolutionary dynamics (Box 2).
124 While we focus on dormancy and the evolution of microbial populations, we believe that the framework and conclusions apply to organisms across the tree of life
126

POPULATION GENETIC CONSEQENCES OF DORMANCY

128 Seed banks preserve genetic and phenotypic diversity by stratifying the population. In a population with a seed bank, individuals can effectively be grouped into active and dormant sub-
130 populations, where they enter and exit a dormant state in a manner analogous to migration between sub-populations (Lennon & Jones, 2011; Blath et al., 2015) (Fig. 1). Seed banks are
132 sometimes viewed simply as an evolutionary buffer (Koopmann et al., 2016). Dormancy preserves existing genetic diversity by decreasing the rate that genetic diversity is removed from
134 the population (Hairston & De Stasio, 1988; Vitalis et al., 2004; Koopmann et al., 2016) and increasing in the effective size of the population (Nunney, 2002). However, when individuals
136 remain dormant for extremely long periods of time, dormancy can have complex and non-intuitive effects on evolutionary dynamics (Blath et al., 2015). In the following sections, we use

138 population genetic theory to examine how seed banks affect each of the fundamental forces of evolution and expand on that theory through the use of novel simulations.

140

Genetic drift — The amount of genetic diversity that can be maintained in an ideal population of

142 finite size is determined by the number of individuals in the population (N) and the per-generation mutation rate (μ). When the rate that genetic diversity is acquired by mutation is

144 equal to the rate that it is lost by drift (i.e., mutation-drift equilibrium), our expectation for the maximum amount of genetic diversity that can be maintained in a population of haploid

146 individuals is $\theta = 2N\mu$. This equation can conveniently be interpreted as the ratio of the rate that genetic diversity is acquired by mutation (2μ) and lost by drift ($1/N$) each generation (Kimura,

148 1969). However, our expectation needs to be modified when a population contains dormant individuals. Because dormant individuals do not reproduce they are not subject to random

150 genetic drift, which increases the maximum amount of genetic diversity that can be maintained in a finite population. Thus, all else being equal, dormancy should inflate estimates of genetic

152 diversity (Levin, 1990).

Two classes of theoretical population genetic models have been used to explore how

154 genetic diversity scales with dormancy. The first class of models examines what is known as the weak seed bank effect. It assumes that individuals can spend a maximum number of generations

156 in the seed bank that is smaller than the number of active individuals in a population, meaning that individuals enter and exit the seed bank before the genetic diversity in the active portion of

158 the population turns over due to the joint effects of mutation and drift (Kaj et al., 2001). The weak seed bank model predicts that dormant individuals increase genetic diversity, but do not

160 change the pattern of ancestry among individuals in the population (i.e., the shape of the

population's genealogy). In contrast, the strong seed bank model has no constraint on the maximum number of generations that an individual can remain in the dormant state (González-Casanova et al., 2014; Blath et al., 2015; 2016). This lack of constraint means that the length of time that an individual spends in the seed bank (where time is scaled as generations) can be longer than the number of active individuals in the population. Under this scenario genetic diversity within the active portion of the population effectively turns over before an individual in the seed bank has the opportunity to reproduce. The result of the strong seed bank effect is that dormancy drastically alters the pattern of ancestry among individuals in the total population in addition to increasing the maximum amount of genetic diversity that can be maintained in the population (Fig. 3a). Here the expected amount of genetic diversity ($\mathbf{E}[\pi]$) in a population with *M* dormant individuals is $\mathbf{E}[\pi] = \theta + \theta(\frac{M}{N})$, which can be interpreted as the amount of genetic diversity expected in a population with *N* actively reproducing individuals ($\theta = 2N\mu$) plus the seed bank's contribution towards reducing the rate of genetic drift scaled by the relative excess of dormant individuals ($\frac{M}{N}$).

In addition to increasing genetic diversity in a population, our simulations suggest that the average length of time required for a population to reach mutation-drift equilibrium drastically increases under the assumption of a strong seed bank (Fig. 3b). However, while the weak and strong seed bank models present viable hypotheses about how dormancy can increase genetic diversity, little work has been done to determine if inferred patterns of ancestry in actual populations capable of entering a dormant state resemble predictions from the strong or weak seed bank effect. Determining whether natural populations capable of entering a dormant state exhibit the strong or weak seed bank effect will require comparing inferred patterns of ancestry to the predictions of each model.

184

Mutation **—** Mutations are the ultimate source of genetic diversity. In populations composed
186 entirely of reproducing (i.e., active) individuals, the majority of newly acquired genetic variation is due to genome replication errors acquired during cell division (Kunkel, 2004). Given that
188 dormant individuals do not reproduce, it is reasonable to expect that all observed genetic diversity was acquired at some point in the past when the population was active, an assumption
190 that is often made in population genetic models that include dormancy (Vitalis et al., 2004; Blath et al., 2015). However, mutations can still arise in populations that experience sub-optimal
192 conditions. For example, the rate of mutation can be effectively decoupled from the rate of replication in *Mycobacterium tuberculosis* due to oxidative DNA damage that occurs when
194 individuals spend a prolonged period of time in a dormant state (Ford et al., 2011; 2013). This reduced correlation between the rate of replication and the rate of mutation via oxidation-induced
196 DNA damage likely applies to other types of mutagens (e.g., high intracellular concentrations of a chemical or physical mutagen). If acquiring mutations during dormancy is common among
198 microorganisms, then it is necessary to consider the rate of mutation that occurs between replication events as well as the mutation rate during replication to understand how genetic
200 diversity is acquired in populations.

The extant that mutation can be decoupled from replication depends on the mechanism
202 used to enter dormancy. For example, DNA is protected during dormancy in spore-forming taxa like *Bacillus* by the extremely low water content in the spore coat and small, acid-soluble
204 proteins that bind to the DNA (Setlow, 1992; 2006; Moeller et al., 2009; 2014), as well as the up-regulation of DNA repair during the early phases of resuscitation (Lenhart et al., 2012; Ramírez-
206 Guadianaa et al., 2012). These mechanisms protect spores from the loss of guanine due to

depurination as well as UV radiation-induced pyrimidine dimers (Setlow, 1992), suggesting that
208 spores acquire mutations at a reduced rate. However, many taxa enter and exit dormancy without
producing specialized resting structures (e.g., spores, cysts, akinetes). In contrast, non-
210 sporulating taxa can likely only rely on mismatch repair mechanisms that are up-regulated during
resuscitation (Mizrahi & Anderson, 1998; Rittershaus et al., 2013) as well as on DNA repair
212 mechanisms that can be maintained under an energy-limited state (Gong et al., 2005; Dieser et
al., 2013; Rittershaus, 2013). For non-sporulating taxa that spend extended periods of time in an
214 energy-limited state it is likely that the long-term mutation rate is primarily determined by the
fidelity of DNA repair mechanisms that operate during dormancy, rather than that of
216 polymerases used during periods of rapid growth. Maintained DNA repair may be a better
mechanism for long-term cell survival rather than an absolute lack of metabolic activity, as
218 evidence of DNA repair has been found in bacteria isolated from half-million-year-old
permafrost samples (Johnson et al., 2007). While the exact cost of maintaining DNA repair
220 mechanisms relative to the total energy budget of energy-limited cells is not known, it is small
enough that it has been ignored in estimates of the cost of cell maintenance under conditions of
222 homeostasis (Lynch & Marinov, 2015) and extreme energy limitation (Kempes et al., 2017),
suggesting that a certain level of mismatch repair can be maintained over long periods of
224 dormancy without draining the total cellular budget.

Attempting to reproduce under sub-optimal conditions, rather than going dormant, can
226 elevate the rate of mutation in a population. Microbial mutation rates are often elevated under
stressful conditions due to the up-regulation of error-prone machinery used for DNA replication
228 and repair (Witkin, 1976; Foster, 2007). It has been argued that this error-prone machinery may
be beneficial under times of stress, as an increased mutation rate should increase the number of

230 beneficial mutations acquired over a given length of time (Rosenberg, 2001; Foster, 2007;
Galhardo et al., 2007). For example, DNA polymerases used under times of stress can confer a
232 competitive advantage to starved populations of *Escherichia coli*, known as the Growth
Advantage at Stationary Phase (GASP) phenotype (Finkel, 2006). However, this temporary
234 fitness advantage can have long-term consequences for the survival of the population. The
majority of mutations are deleterious (Lynch et al., 1999) and there is no reason to expect that an
236 increased mutation rate will change the distribution of mutational fitness effects. Thus, any
increase in the mutation rate will likely be accompanied by a proportional increase in the number
238 of deleterious mutations acquired per unit time, contributing towards the long-term deterioration
of the genome (Gerrish et al., 2013; Lynch et al., 2016). Populations that continue to grow in a
240 starved state likely constitute an evolutionary dead end, as the accumulated deleterious mutations
would result in a decrease in fitness once the environment improves. Rather, if there is sufficient
242 temporal variation in the environment then it is possible that the long-term geometric fitness of a
population would be maximized by persisting in a dormant state rather than attempting to
244 reproduce and incurring the negative effects of an elevated mutation rate.

246 ***Selection*** — Because dormancy can act as a buffer against the stochastic forces of mutation and
genetic drift, it is reasonable to expect that the presence of a seed bank will reduce the rate that
248 the directional force of natural selection removes genetic diversity from the population. Natural
selection is less efficient in models that assume a weak seed-bank effect, where the average
250 amount of time required for selection to drive a beneficial allele to fixation (T_{fix}) increases
quadratically with the average number of generations that an individual spends in a dormant state
252 (Koopmann et al., 2016). However, little work has been done to examine selection under a strong

seed-bank effect or how selection affects T_{fix} differently for active and dormant portions of the population. Intuitively we would expect that T_{fix} would increase, since individuals can spend an even longer period of time in a dormant state. Consequently, beneficial alleles should take a longer period of time to go to fixation because individuals must enter and exit the seed bank to spread the beneficial allele throughout the whole population. To test this hypothesis, we simulated the trajectory of a beneficial allele in a population subject to the strong seed bank effect and examined the dormant and active portions of the population separately (see Supplemental Materials: Selection simulation). As expected, we found that the average amount of time required for a beneficial allele to reach fixation increases with the average number of generations that an individual spends in the seed bank. Our simulations are supported by previously developed theory that found a similar increase in T_{fix} as the average number of generations that an individual spends in a dormant state increases (Fig. 4a,b) (Koopmann et al., 2016). However, because the seed bank stratifies the population into active and dormant components and a fitness advantage only increases the frequency of a beneficial allele if its carrier is reproducing, it is necessary to examine the active sub-population separately from the dormant sub-population. In our model, the beneficial allele initially rose in frequency until it was effectively fixed (a.k.a., quasi-fixation), where it then fluctuated below a frequency of one as individuals continued to exit and enter the seed bank (analogous to back-mutation in a single-locus model). By examining the active portion of a population with a seed bank in a simulation of single locus selection, we found that the average number of generations required for a beneficial allele to reach quasi-fixation is similar to that for the dormant portion up until the average time in the seed bank approaches 100 generations, which is the threshold between the weak and strong seed bank effects (Fig. 4b). Surprisingly, the average length of time until a

beneficial allele is quasi-fixed among actively reproducing individuals decreases after the strong seed bank threshold, approaching the expected T_{fix} for a population without a seed bank. This decrease in T_{fix} is likely because the input of genetic diversity via resuscitation is low enough that it does not increase the amount of time required for a beneficial allele to reach fixation, a novel result that has not previously been reported. Contrary to expectations, our simulations suggest that if the average length of time that an individual spends in a dormant state (scaled as generations) is longer than the number of active individuals, the presence of a seed bank will not interfere with selection in the active portion of the population.

The decline of T_{fix} for the active portion of a population subject to the strong seed bank effect has important implications for the long-term evolutionary dynamics of microbial populations. Because the majority of a given microbial genome is likely clonal, the rate of evolution in a population undergoing adaptive evolution can be sufficiently described by the beneficial mutation rate (U) and the strength of selection operating on a population of constant size (N) (Desai et al., 2007). Given that the average time it takes for a beneficial mutation to arise (T_{mut}) is much greater than T_{fix}, the population will evolve at a rate (v), which is proportional to the rate that beneficial mutations of small effect arise and fix within a population (i.e., $v \propto s^2NU$) (Park et al., 2010). If this condition is violated, multiple beneficial mutations can arise on different genetic backgrounds and compete in the population, increasing T_{fix} through the process of clonal interference (Gerrish & Lenski, 1998). As no one has examined clonal interference in an asexual population with a seed bank, there is no theory to draw on that would allow one to determine whether dormancy increases or decreases the rate of evolution in the multiple mutation regime. Our results suggest that the rate of evolution in the active portion of a microbial population with a strong seed bank can likely be approximated as a series of mutations fixing in

the population. However, T_{fix} will increase if individuals on average spend fewer generations in
300 a dormant state than the number of active individuals (Fig. 4b). If the presence of a seed bank
increases T_{fix} to the point that it is not much larger than T_{mut}, then multiple beneficial mutations
302 will segregate simultaneously, moving the population into the clonal interference regime. To
fully describe the dynamics of clonal interference in a population with a seed bank it will be
304 necessary to extend the single beneficial mutation dynamics described here to the multiple
mutation regime using simulations and population genetic theory.

306 The average amount of genetic diversity among all individuals in a population with a
seed bank will be preserved for longer periods of time in the face of selection. This preservation
308 of genetic diversity has important implications for microbial evolution, as it is often thought that
microbial populations contain low levels of diversity across their genomes due to linked positive
310 selection (i.e., genetic draft) (Gillepsie, 2000; Lynch, 2007). Genetic draft can dominate genetic
drift as the primary stochastic force in large microbial populations, constraining the maximum
312 amount of genetic diversity that can be maintained in a population (Smith & Haigh, 1974;
Gillespie, 2000). Evolution experiments have confirmed that linked selection regularly reduces
314 genetic diversity in microbial populations and that it operates on the relatively fast time scale of
hundreds to thousands of generations (Maddamsetti et al., 2015; Tenaillon et al., 2016).
316 However, such experiments are commonly carried out in constant environments that are near-
optimal for growth and reproduction, potentially providing an inaccurate representation of how
318 selection operates in naturally occurring populations where individuals can enter a dormant state.
While it is difficult to examine the genetic diversity of natural microbial populations over time, a
320 recent study found evidence of only a single case of genome-wide linked selection in 30
freshwater microbial populations monitored over nine years (Bendall et al., 2015). Given that

322 approximately half of all bacterial cells in freshwater lakes may be dormant (Lennon & Jones, 2011), the small number of selective sweeps observed could be explained by the presence of a
324 seed bank that increased the length of time required for selection to drive beneficial alleles to fixation among both active and dormant individuals. Accurately inferring the extant that
326 selection can act on a population that capable of entering a dormant state requires accounting for the seed bank.

328

Recombination — Dormancy can likely preserve the diversity of genes as well as the diversity
330 of alleles generated by recombination. The ability for microorganisms to acquire and exchange DNA by horizontal gene transfer (HGT) is a major evolutionary feature that can promote the
332 spread of beneficial genes within a population (Medini et al., 2005; Lapierre & Gogarten, 2009; Dixit et al., 2016). The stochastic processes of gene gain and loss via HGT leads to the uneven
334 distribution of genes among genomes in a population (Berg & Kurland, 2002; Baumdicker et al., 2012). This uneven distribution is often summarized as the total number of genes found within
336 the population (i.e., the pangenome) and the number of genes shared among all individuals within the population (i.e., the core genome). Similar to how the presence of a seed bank
338 increases the maximum amount of genetic diversity that can be maintained in a finite population by reducing the effect of genetic drift, it is possible that the presence of a large seed bank would
340 increase the size of the pangenome. In addition, a strong seed bank could alter the distribution of gene frequencies within a population, analogous to how the strong seed bank effect alters the
342 distribution of allele frequencies within a population (Blath et al., 2015). Given that closely related microorganisms can often share very little gene content, it is possible that the ability for

344 microorganisms to persist in a dormant state can preserve genic diversity acquired by recombination.

346

Gene flow **—** Seed banks may reduce the effect that migration has on altering allele frequencies

348 within a population (i.e., gene flow). The ability to enter a dormant state can increase the chance that any two individuals share a common ancestor within a population (i.e., the probability of

350 identity by descent) and reduce the amount of estimated differentiation among populations (i.e., the fixation index, F_{st}) (Vitalis et al., 2004; Živković & Tellier, 2012; Hollander & Pederzani,

352 2017). This increase in genetic similarity due to the presence of a seed bank has been found in natural populations of plants (Tellier et al., 2011; Živković & Tellier, 2012). However, dormancy

354 and migration are not always independent. For many organisms, dormancy can increase the probability of successfully migrating and establishing in a new population. For example, the

356 spatial distribution of endospore-forming thermophilic bacteria in marine sediments closely reflects global ocean currents (Müller et al., 2014). However, dormancy and migration can

358 represent a trade-off if the ability to enter a dormant state involves investing energy into a different mechanism than the one used to migrate (Olivieri, 2001). While the potential for

360 dormancy and migration to be either positively or negatively correlated is well known in life history theory, little work has been made towards accounting for this relationship in population

362 genetic theory. To understand the evolutionary consequences of dormancy and migration it is necessary to examine how these life history strategies interact to shape patterns of shared genetic

364 diversity between populations.

366 **MACROEVOLUTIONARY CONSEQUENCES OF DORMANCY**

In the previous section, we examined how seed banks influence the rate that allele frequencies change over relatively short periods of time within a lineage (i.e., microevolution). However, if seed banks buffer the aggregated effects of multiple evolutionary forces over long periods of time, then dormancy may have important implications for macroevolutionary phenomena. In the following section, we examine how dormancy influences processes and patterns such as the rate of molecular evolution, shared ancestry among lineages, speciation, and extinction.

The rate of molecular evolution **—** If dormancy can affect the rate of evolution in microbial populations, we would likely observe it in bacteria that are uniquely capable to persist for extended periods of time in an inactive state via their capacity to form resistant, long-lived spores. Many bacteria in the phylum *Firmicutes* possess the ability to form endospores, which are thought to be one of the most metabolically inert forms of life on the planet (Setlow, 2014). Endospore formation is triggered by low resource availability, where the endospore is built within the cell, lyses, and is released into the environment (Tan & Ramamurthi, 2013). These endospores are persistent and in some extreme instances have reportedly been found to survive for hundreds of millions of years (Vreland, 2000). Endospores can endure exposure to extreme conditions such as high doses of gamma and UV radiation (Nicholson et al., 2000; 2005), desiccation (Nicholson et al., 2000), and the vacuum of space (Horneck et al., 1994; 2012). Given that spore-forming bacteria can persist for long periods of time without reproducing, one might expect non-spore-forming relatives to have more rapid rates of molecular evolution. This hypothesis was tested by analyzing a large collection of *Firmicutes* genomes. Some of these genomes came from isolates that had the ability to form endospores while other genomes came

390 from isolates that had lost the ability to form endospores (Weller & Wu, 2015). The rate of
amino acid and synonymous substitutions for non-spore forming isolates were significantly
392 elevated relative to spore-forming taxa and the phylogenetic branch length declined as the
number of spore-forming genes within a genome increased (Fig. 5a; Weller & Wu, 2015). These
394 results suggest that the rate of evolution increases when a lineage loses the ability to enter
dormancy via sporulation. To further evaluate this effect of dormancy on the rate of evolution,
396 we ran a simulation for the simple case of a neutrally evolving population, where we
manipulated the average number of generations that an individual spends in the seed bank and
398 calculated the substitution rate after 10,000 generations (see Supplementary Methods for more
detail). For the entire population (i.e., both active and dormant individuals) we found that
400 substitution rate declined as average time in the seed bank increased, a finding that should apply
to microbial evolution irrespective of the mechanisms controlling transitions into and out of a
402 dormant state (Fig. 5b).

While theoretical and empirical evidence suggests that seed banks can reduce the rate of
404 evolution within a lineage, this effect may be short lived if the ability to enter and exit a dormant
state cannot be maintained. For example, the ability to form endospores has been lost multiple
406 times within the *Firmicutes* phylogeny (Weller & Wu, 2015). The repeated loss of this seemingly
beneficial trait across lineages can be explained by adaptive and non-adaptive evolutionary
408 processes operating within a lineage. Organisms often need to invest in morphological or
physiological structures that allow individuals to enter and exit a dormant state in response to
410 fluctuating environmental conditions (Ayati & Klapper, 2012; Akiyama et al., 2017). The
selective advantage of maintaining the ability to enter a dormant state depends on both this
412 energetic cost as well as the long-term fitness contribution of dormancy in a fluctuating

environment, where the strength and direction of natural selection can change over time (Simons,
414 2009). In relatively stable environments, the mechanism necessary to enter a dormant state may
be lost from a lineage, either due to relaxed selection leading to the accumulation of mutations at
416 dormancy-encoding loci or selection against the energetic cost of the mechanism (Dawes &
Thornley, 1970). For example, in long-term evolution experiments, five populations of *Bacillus*
418 *subtilis* lost the ability to form endospores when medium was continuously supplied so that
individuals did not experience starvation (Maughan et al., 2007). The accumulation of mutations
420 due to relaxed selection was responsible for the loss of endospore formation in four of the five
populations, while the loss of sporulation actually increased fitness in the other population. Spore
422 formation may be lost because it is a trait that is encoded by more than 200 genes (Galperin et
al., 2012), making it a large target for mutation. Alternatively, it is possible that the underlying
424 genes may be lost because the energetic cost of even a single nucleotide is visible to natural
selection in bacteria (Lynch & Marinov, 2015). In contrast to endospore-formation, other
426 mechanisms regulating dormancy appear to require fewer genes than what is reported in spore-
forming bacteria. For example, resuscitation promoting factor (Rpf) is an enzyme encoded by a
428 single gene that terminates dormancy for many bacteria belonging to the *Actinobacteria* phylum
(Mukamolova et al., 1998; Keep et al., 2006). Rpf cleaves the 1-4 linkage between the amino
430 sugars N-acetylglucosamine and N-acetylmuramic acid in peptidoglycan, a major cell-wall
component of nearly all bacteria. The gene encoding Rpf likely has a lower probability of being
432 lost owing to its small size and lower energetic cost. Ultimately, whether a mechanism used to
enter a dormant state is retained in a lineage long enough to affect the rate of evolution will
434 depend on the amount of environmental variation the lineage experiences over extended periods

of time, the energetic cost of the mechanism, the mutation rate of the lineage, and the number of
436 nucleotides that encode the mechanism.

438 ***Shared ancestry among lineages*** — Dormancy may provide novel insight into confusing patterns of ancestry among microbial lineages. Many microbial genomes harbor genes that have
440 no detectable homologues in closely related lineages (i.e., ORFans) (Daubin & Ochman, 2004; Mira et al., 2004; Zhaxybayevaa et al., 2009). One explanation for the existence of ORFans is
442 horizontal gene transfer (HGT). HGT is a reasonable hypothesis given that genic and genomic content can vary among and within microbial lineages due to the exchange of genetic material
444 (Ochman et al., 2000). However, certain ORFans are essential for the process of cell division, even though they are not present in neighboring lineages (e.g., Mira et al., 2004; Zhaxybayevaa
446 et al., 2009). Therefore, it is unclear to what extant essential genes can be lost and acquired without resulting in the death of the cell. Another explanation for existence of ORFans is seed
448 banks (González-Casanova et al., 2014). If some fraction of individuals can remain dormant for extremely long periods of time (González-Casanova et al., 2014), then the active portion of the
450 population may continue to accumulate substitutions to the point that certain genes in the active pool no longer share homology with members in the dormant pool. To test this, a set of ORFan
452 genes from the nitrogen-fixing soil bacterium *Azotobacter vinelandii* and neighboring lineages were examined (González-Casanova et al., 2014). These ORFan genes had the characteristic
454 codon-usage and GC-content biases of *A. vinelandii*, but showed little homology to closely related members of the genus *Pseudomonas*, a result that was interpreted as support for the
456 strong seed bank hypothesis.

While the strong seed bank hypothesis may explain the long-term evolutionary consequences of remaining in a dormant state, an alternative hypothesis can explain the existence of ORFans. First, additional copies of essential genes could be acquired by HGT, leading to relaxed selection on each gene copy. The original copy could then be physically lost due to gene deletion or functionally lost due to accumulated mutations (Lynch, 2007). This new copy would then gradually acquire the same GC-content as the rest of the genes within the lineage through a combination of lineage-specific mutation bias and the rate of GC-biased gene conversion (Lynch, 2007; Lassalle et al., 2015). In addition, lineage-specific selection on translational efficiency would shape the codon composition of the gene (Drummond & Wilke 2008; Plotkin & Kudla, 2011), particularly if the lineage has a large effective population size (Sharp & Li, 1987; Sharp et al., 2005). These directional pressures suggest that horizontally acquired genes that are retained in the genome may lose their signal of homology while becoming similar in composition to vertically acquired genes. While the theoretical models necessary to describe the effect of the seed bank on the genetic composition of microbial lineages exist (González-Casanova et al., 2014), further study is needed to determine whether the strong seed bank effect applies to gene-specific patterns of ancestry.

Speciation — While not theoretically well-developed, it is possible that dormancy can affect rates of speciation. Population genetic theory suggests that the presence of a seed bank can preserve genetic similarity among separate populations (Vitalis et al., 2004). The ability to exit a dormant state effectively acts like gene flow in physically separated populations, preserving genetic diversity and similarity that might otherwise be lost due to selection acting on newly arisen alleles within a population. The preserved genetic similarity due to the seed bank would

480 likely slow the rate of evolutionary divergence between lineages due to geographic divergence,
reducing the rate of allopatric speciation. However, if a portion of the population remains in a
482 dormant state while the rest of the population diverges to the extent that genetic material can no
longer be exchanged, then dormant and active individuals may ultimately diverge into separate
484 lineages. This process of divergence is an extension of the strong seed bank effect previously
discussed (González-Casanova et al., 2014), where the focus is on how evolutionary divergence
486 between dormant and active individuals can prevent the exchange of genetic material, rather than
on patterns of ancestry. While seed banks may influence the rate of speciation, very little is
488 known about how microbial lineages split or the rate that they split, regardless of the microbial
species definition. While the rates of speciation are unclear, recent research on the net difference
490 of speciation and extinction rates (i.e., diversification) in microorganisms suggests that microbial
taxa split at a constant rate through time (Marin et al., 2017). If so, then we would expect that
492 any decrease in the rate of speciation due to the presence of a seed bank would be associated
with a similar increase in the rate of extinction, or vice-versa. The exact evolutionary
494 mechanisms that are responsible for this constant rate of lineage diversification are currently
unknown. Determining to what extant the ability to persist in a dormant state reduces the rate of
496 speciation requires further research.

498 ***Extinction*** — Because seed banks can maintain preserve the size of the population, they can
likely buffer populations from extinction (Kalisz & McPeek, 1993). However, while next to
500 nothing is known about extinction rates in microorganisms (Weinbauer & Rassoulzadegan,
2007), we can extend evolutionary models of microbial extinction to examine how the ability to
502 enter a dormant state might decrease the rate of extinction. The primarily clonal structure of

microorganisms means that most mutations cannot be removed by recombination once they
504 become fixed. It is often argued that the rate of extinction in a microbial lineage is determined by the fixation rate of deleterious mutations, since once a deleterious mutation is fixed it can only be
506 removed by the fixation of a reverse mutation at the same site; a process known as Muller's Ratchet (Muller, 1964; Felsenstein, 1974). The negative fitness effect of deleterious mutations
508 accumulated over time can lead to the extinction of a lineage. In this case, extinction happens because reproductive output is reduced due to fixed deleterious mutations, increasing the
510 strength of genetic drift relative to natural selection, leading to a positive feedback loop where deleterious mutations continue to accumulate and reproductive output declines over time. This
512 feedback loop eventually results in the extinction of the lineage, a phenomenon known as a mutational meltdown (Gabriel et al., 1993; Lynch et al., 1993). Because lineages persisting in a
514 dormant state likely fix mutations at a far lower rate (Fig. 5), extinction via mutational meltdown is less likely to occur within lineages capable of forming a seed bank. Given that dormant
516 individuals can persist on time scales upwards of millions of years (Cano et al., 1995; Greenblatt et al., 2004), it is worth determining whether there is a relationship between the age of a
518 microbial lineage and its ability to persist in a dormant state.

520 **CONCLUSION**

The ability to enter a dormant state is a common life history strategy that is found across
522 the tree of life. Historically dormancy has been viewed as an adaptive trait that preserves genetic and taxonomic diversity, with comparatively little attention given to its effects on the
524 fundamental forces that underlie evolution. Using recent developments in theoretical population genetics and novel simulations we examined how the ability to enter a dormant state effects the

population genetic forces that underlie evolutionary biology as well as common estimates of genetic diversity. Specifically, we identified the demographic scenarios where dormancy can affect natural selection. We then extended these results to determine how dormancy can influence the evolutionary dynamics of microbial lineages and in what environmental systems the ability to enter a dormant state is a viable life history strategy. We conclude that while dormancy can influence evolutionary dynamics and common estimates of genetic diversity, it is necessary to consider whether dormant organisms within a system are engaging in a life history strategy or simply reducing their metabolic rate out of physiological necessity. By considering the short-term population genetic and long-term macroevolutionary implications of dormancy as an adaptive trait researchers can examine the evolutionary importance of dormancy. Determining the extant that dormancy influences microbial evolution will require empirical research on whether dormant microorganisms in the environment are engaging in a life history strategy as well as continued development of the population genetic theory that accounts for the evolutionary effects of dormancy.

Box 1: Dormancy in low-energy environments

In nature, most organisms experience conditions that are suboptimal for growth and reproduction. This is true for microorganisms, just as it is for plants and animals. For example, approximately 30-50% of the prokaryotic (i.e. Bacteria and Archaea) cells on Earth live under continents and on the ocean floor in a marine subsurface habitat known as the "deep biosphere". In this environment, microorganisms are fueled by remnants of organic matter produced in the well-lit, productive surface waters that sink to the bottom of the ocean. As a result, microorganisms in the deep biosphere rest on the thermodynamic edge of life and death (Kallmeyer et al., 2012; Parkes et al., 2014; Jørgensen & Marshall, 2016; Starnawski et al., 2017). The deep biosphere likely constitutes the largest seed bank on the planet, where spore-forming bacteria are one of the most dominant groups of microorganisms (Lomstein et al., 2012). Furthermore, microorganisms in the deep biosphere spend an extremely long time in the seed bank. Based on rates of amino acid racemization, it is estimated that the microbial biomass pool may only turnover once every thousand years (Lomstein et al., 2012; Hoehler & Jørgensen, 2013; Jørgensen & Marshall, 2016). In a recent study where four microbial lineages within the deep biosphere were sequenced, all lineages showed little-to-no signature of divergence or difference in standing genetic variation from populations at the surface. In addition, the ratio of synonymous to non-synonymous polymorphisms did not change with sediment depth for three out of four lineages, suggesting that populations within the deep biosphere are not undergoing adaptive evolution as they sink deeper into the sediment (Starnawski et al., 2017). These findings are consistent with expectations of how dormancy should affect evolution as a buffer, suggesting that the lack of divergence, adaptive evolution, and similar estimates of standing genetic variation across sediment depth could be explained by the presence of a large microbial seed

572 bank. However, certain areas of the deep biosphere are thought to be remarkably stable for long
periods of time, perhaps millions of years (Jørgensen, 2012), a length of time that is likely
574 several orders of magnitude longer than the average generation time. However, the slow turnover
rate raises the question of whether dormancy is a viable life history strategy for microorganisms
576 in the deep biosphere. Without a change in environmental conditions which would occasionally
favor growth and reproduction, the deep biosphere may simply reflect a very large collection of
578 microorganisms that are on a slow march to death. Without the possibility of resuscitation,
remaining in a dormant state is ultimately an evolutionary dead end. These stable environmental
580 conditions suggest that dormancy is unlikely to be a viable life history strategy (Malik & Smith,
2008). Rather, in the deep biosphere dormancy may simply be a metabolic necessity due to the
582 energetic constraints of the environment.

In contrast, dormancy is likely a viable life history strategy in certain regions of the
584 permafrost, a temperature and energy-limited environment covering 25% of Earth's terrestrial
surface (Graham et al., 2012). Upper layers of the permafrost that are not covered with ice can go
586 through annual freeze-thaw cycles, providing the temporal environmental variation necessary to
favor dormancy as a life history strategy (Cohen, 1966; Malik & Smith, 2008). These freeze-
588 thaw cycles could produce boom-and-bust periods of population growth that would leave a
generation-time effect on the rate of molecular evolution, where lineages closer to the poles
590 spend more time on average in a dormant state and show a reduced rate of molecular evolution.
If so, this might result in latitudinal patterns of diversification, which has been a long-standing
592 pattern of diversity in a range of biological systems. However, many microorganisms within the
permafrost may not be completely dormant. For example, psychrophilic taxa isolated from the
594 permafrost are capable of genome replication at temperatures as low as -20 °C (Tuorto et al.,

2014) and evidence of DNA repair has been found in taxa that have persisted in the permafrost
596 for hundreds of thousands to millions of years (Johnson et al., 2007; Dieser et al., 2013). Unlike
the deep biosphere, an appreciable fraction of the microorganisms within the permafrost have
598 likely adapted to grow and reproduce in their environment. However, even with reproduction in
the permafrost, a generation-time effect would still be possible if generation time varied
600 seasonally. In addition, evidence of DNA repair does not mean that microorganisms within the
permafrost maintain a high level of metabolic activity. The energetic cost of DNA repair is low
602 (see section on mutation), suggesting that it is possible for cells within the permafrost to maintain
genome integrity in an otherwise metabolically inactive state. However, as the average global
604 temperature increases due to human-induced climate change, the range of the permafrost will
recede (Seneviratne et al., 2012). This rapid directional shift in Earth's climate will likely impose
606 a strong selective pressure against dormancy as a life history strategy and favoring sustained
growth and reproduction as seasonal environmental variation declines.
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618 **Box 2: Dormancy and the evolution of infectious diseases**

620 Pathogens exist in environments that fluctuate rapidly owing to the immune response of the host and the processes of infection and transmission. Microorganisms often experience
622 periods of high-growth during the early stages of infection and low-growth in the later stages of infection and when they disperse outside of the host environment (Oliver et al., 1995). These
624 temporal fluctuations in growth conditions suggest that dormancy would be favored by selection as a life history strategy. For example, *Yersinia pestis*, the causative agent of the plague, often
626 enters a dormant stage that allows it to persist between infections (Pawlowski et al., 2011). This ability to persist is likely an adaptation, as *Y. pestis* contains several highly conserved genes that
628 can contribute towards its survival long after the death of the host (Easterday et al., 2012). There is also evidence that dormancy can contribute towards the survival of a pathogen within the host.
630 Approximately one-third of the world's population is infected with "latent" (i.e., dormant) strains of *Mycobacterium tuberculosis* (Dye et al., 1999), the causative agent of tuberculosis that kills
632 over 1 million people per year (GBD, 2016). In addition to preventing population extinction, the ability to enter a dormant state would decrease the temporal variation in the size of the
634 population, maintaining standing genetic variation that could contribute towards adaptive evolution in a new host. If dormancy is as common as these examples suggest, then the ability to
636 enter and exit a dormant state could be a major feature in the evolutionary dynamics of pathogenic microorganisms.

638 Dormancy may also contribute to the evolution of antibiotic resistant pathogens. The evolution of disease-causing bacteria that are resistant to the most commonly used antibiotics is a
640 major global health care concern (WHO, 2014). Methicillin-resistant *Staphylococcus aureus*

infections alone kill more than 11,000 Americans each year (Gross, 2013; Golkar et al., 2014).
642 Antibiotic resistant infections in general are estimated to kill 10 million people by 2050 (O'Neill, 2014). Dormancy can contribute towards the severity of these infections, as bacterial cells can
644 often survive antibiotic treatment by remaining in a dormant state, contributing towards chronic infections within hosts (Wood et al., 2013; Zhang, 2014). Natural selection can optimize the
646 length of time that bacteria remain dormant under antibiotic treatment (Fridman et al., 2014), suggesting that dormancy contributes towards long-term geometric fitness as a life history
648 strategy. In addition to preventing cell death, bacteria that survive antibiotic treatment can evolve antibiotic resistance, simply because they are alive long enough to acquire a beneficial mutation
650 (Levin-Reisman et al., 2017). As the annual number of deaths due to the persistent infection of antibiotic resistant bacteria continues to increase (WHO, 2014), it will be necessary to develop
652 treatments and strategies to remove reservoirs of dormant bacteria. One potential strategy for targeting pathogens while minimizing the risk of antibiotic resistance is to resuscitate dormant
654 cells alongside a course of antibiotics. The development of treatments designed to resuscitate dormant pathogenic bacteria is in the early stages, but holds promise. For example, in sputum
656 samples of individuals infected with *M. tuberculosis*, 80–99.99% of cells were only detectable after being resuscitated with Rpf (Mukamolova et al., 2010). However, because a large fraction
658 of infections are acquired in hospitals (Klevens et al., 2007), it will be necessary to develop strategies to remove dormant disease-causing bacteria in buildings (i.e., the built environment) as
660 well as in infected hosts. Recent work on characterizing the built environment suggests that the ability to persist is the primary trait that shapes the composition of indoor microbial communities
662 (Gibbons et al., 2015; Gibbons, 2016), a trait found in many disease-causing bacteria. Combating the emergence of antibiotic resistant bacteria and the role of dormancy as an adaptive trait will

664 require re-examining the architecture and materials used to construct the buildings where infection is treated as well as infection treatment itself.

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AUTHOR CONTRIBUTIONS

WRS and JTL conceived of the ideas for the paper; WRS created and executed the simulations; WRS and JTL wrote the paper.

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FIGURE CAPTIONS

1088 **Figure 1:** Transitioning in and out of a seed bank is analogous to the migration of individuals between populations. In **a)** we see a population with a seed bank where individuals switch
1090 between active and dormant states. In **b)** we see two populations where individuals can migrate between each population. An important difference between **a)** and **b)** is that individuals must
1092 originate in the active pool, because dormant individuals cannot reproduce. While dormant individuals can die in this model, their death rate is much lower than that of active individuals.
1094 This conceptual model was first presented in an ecological context (Lennon & Jones, 2011) and later used for the seed bank coalescent (Blath et al., 2015).

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Fig. 2: The single cell metabolic activity distribution in a bacterial population at different time-
1098 points. The bacterium used is a strain of *Janthinobacterium*, an aerobic, Gram-negative, soil-dwelling β-proteobacteria. The blue, yellow, and red histograms represent samples taken at 3, 9,
1100 and 200 hours after the culture was inoculated, respectively. The distribution of metabolic activity depends on the growth state of the population, where the mode decreases as well as the
1102 shape of the distribution as the population enters a dormant state. During the process of resuscitation the mode increases corresponding to an increase in growth rate (see Supplemental
1104 Materials).

1106 **Figure 3: a)** The expected level of nucleotide diversity (**E** $[\pi]$) for a sample of active individuals increases with the number of generations that an individual on average spends in the seed bank
1108 for a population at mutation-drift equilibrium with a given population-scaled mutation rate ($\theta = 2N\mu$). Here N is the number of active individuals, M is the number of dormant individuals, and

1110 μ is the mutation rate for active individuals, where diversity is estimated as $\mathbf{E}[\pi] = \theta + \theta(\frac{M}{N})$ (Blath et al., 2015, eq. 21). The average number of generations that an individual spends in the
1112 seed bank can be modeled as a geometric distribution, where the probability of exiting the seed bank each generation is $\frac{c}{M}$ and c is the number of individuals that exit the seed bank each
1114 generation (Blath et al., 2015; 2016). The vertical grey-dashed line represents the point where the average number of generations that an individual spends in the seed bank is greater than the
1116 number of active individuals in the populations (i.e., the strong seed bank threshold). **b)** The length of time required to reach mutation-drift equilibrium increases with the average number of
1118 generations that an individual spends in the seed bank. These results are from a simulated population evolving over time that is subject only to mutation and drift with a constant seed bank
1120 size. In both **a)** and **b)** We assume that dormant individuals do not acquire mutations. See Supplementary Information for more detail on implementation of model.

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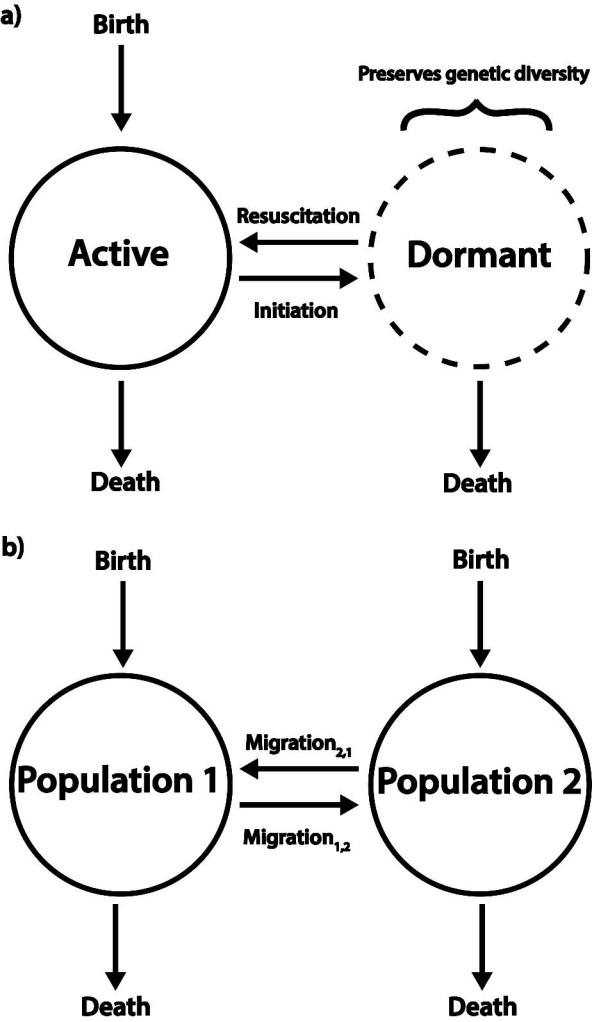
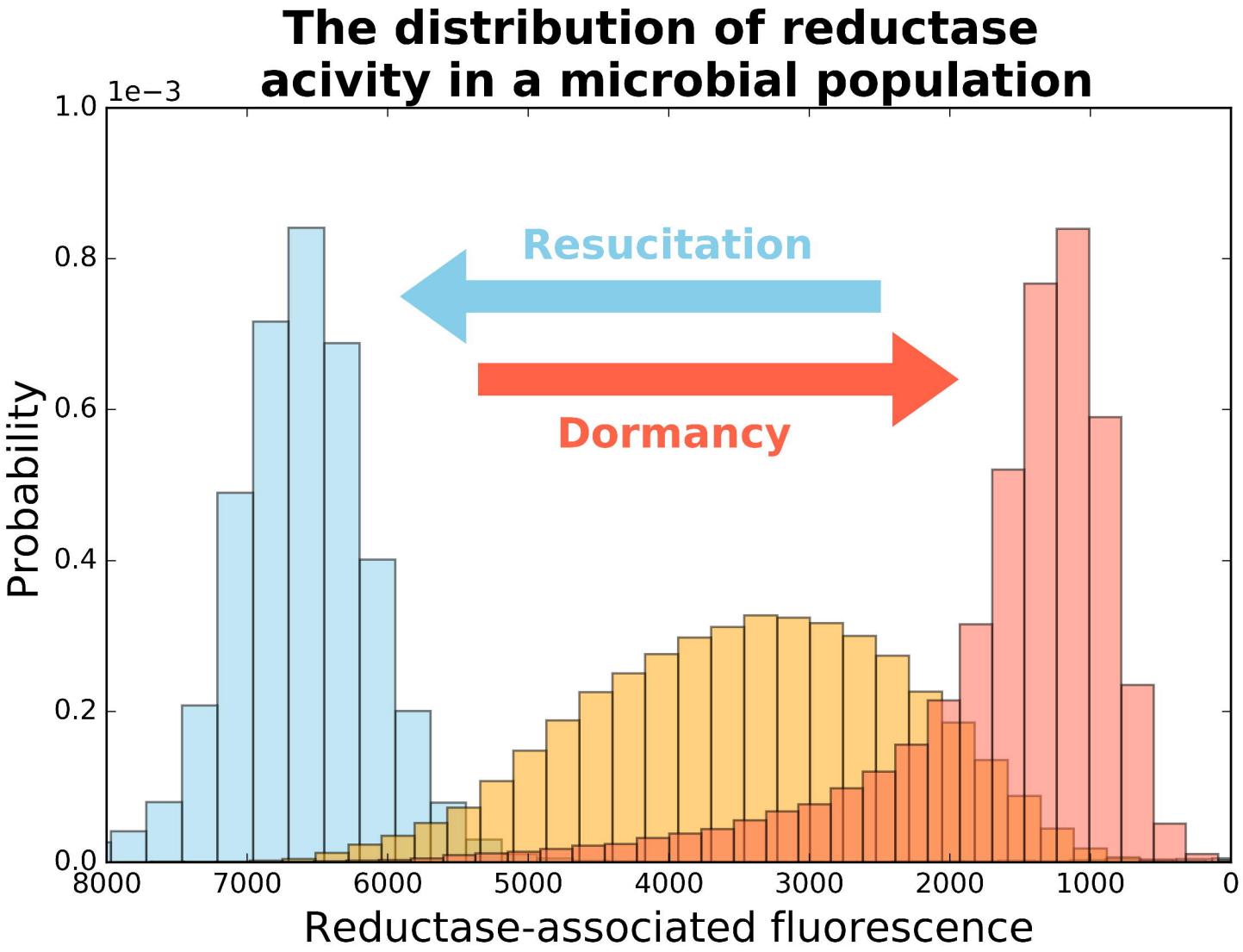
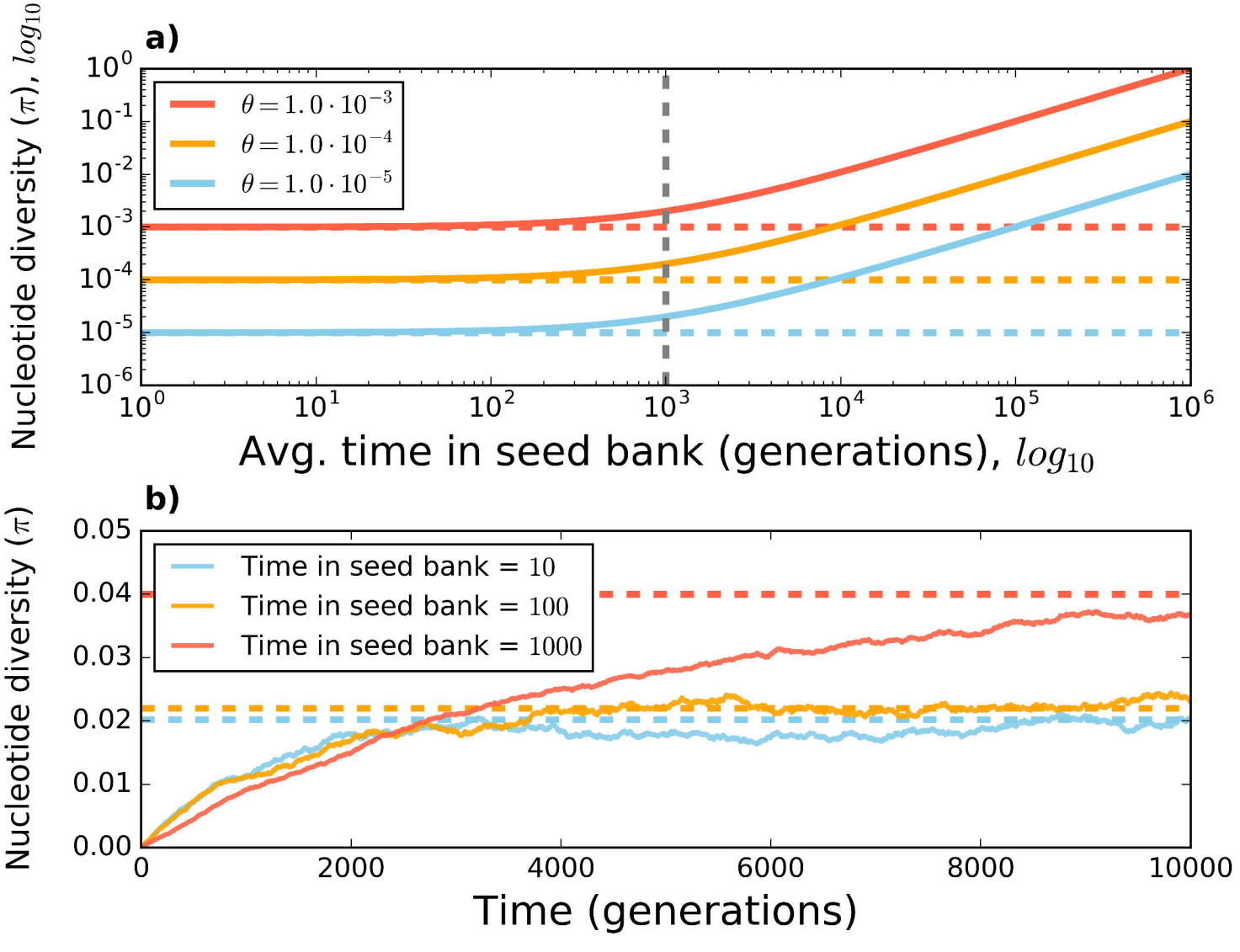
Figure 4: a) The average length of time that an individual spends in a dormant state influences
1124 the trajectory of a beneficial allele destined to go to fixation. The horizontal grey line indicates an allele frequency of 0.5, showing how the beneficial allele first becomes fixed in the active
1126 portion of the population and requires an extended period of time to become fixed in the seed bank as the average length of time spent in a dormant state increases. **b)** The length of time until
1128 an allele is effectively fixed (T_{fix}) for both active and dormant individuals scales with the average number of generations that an individual spends in a dormant state. However, T_{fix} for
1130 the active portion of the population decreases in the strong seed bank regime and eventually approaches the expected time to fixation for an ideal population with the same number of active
1132 individuals and strength of selection (horizontal grey line) (Eriksson et al., 2008, eq. 37). We

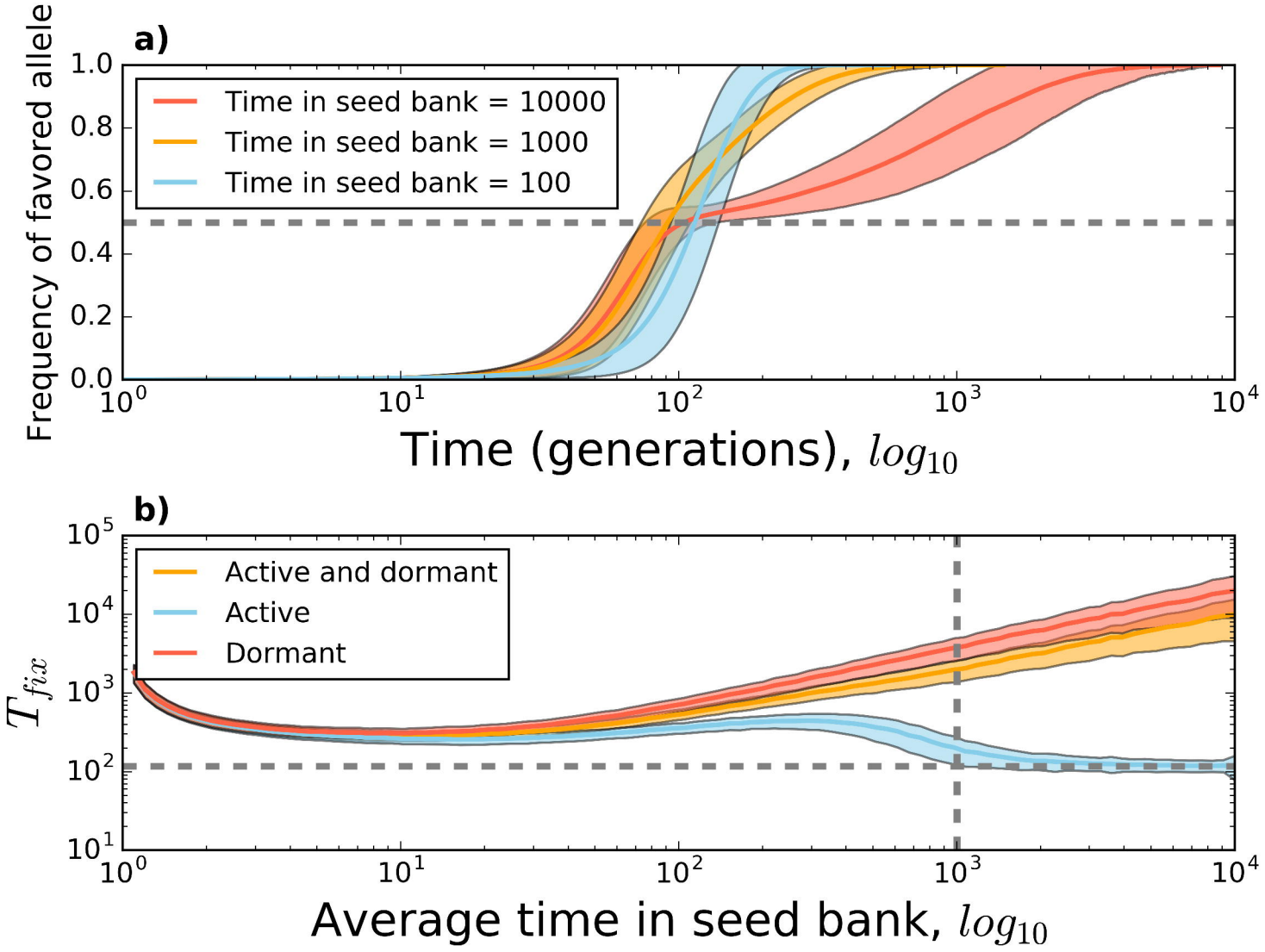
believe that this is because the beneficial allele can reach fixation before dormant individuals with the alternative allele resuscitate, preventing the seed bank from reducing the efficiency of selection. These results for **a)** and **b)** were obtained from a simulated Moran model of a selective sweep with a seed bank (see Supplementary Materials for details).

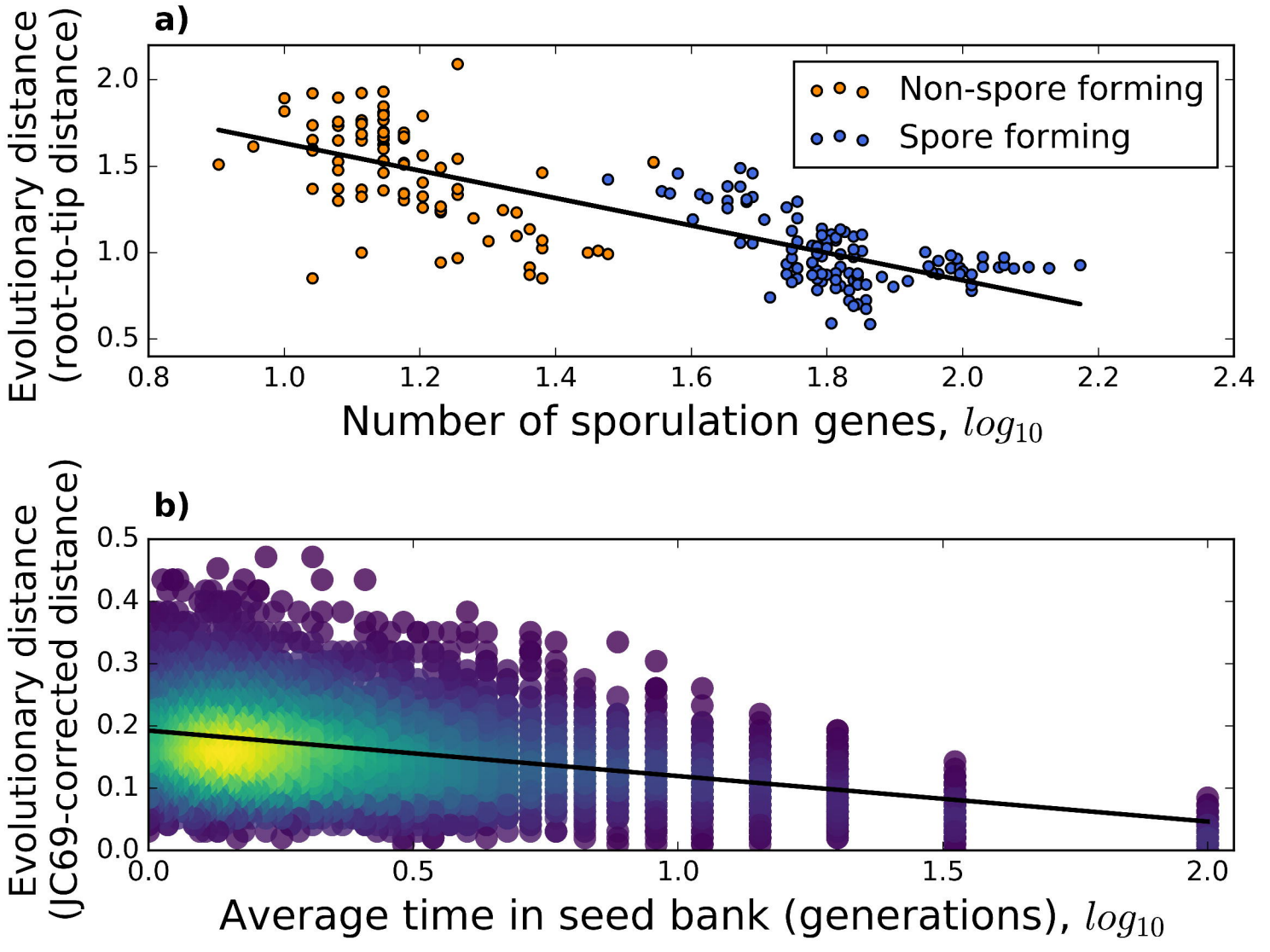
Figure 5: a) The evolutionary distance (calculated as the root-to-tip sum on the branch of the phylogeny) declines as the number of sporulation-associated genes increases among *Firmicutes* taxa, a common group of bacteria found in soils and hosts. The publically available data presented here is from the *Firmicutes* phylogeny of conserved genes (Weller & Wu, 2015). **b)** The evolutionary distance (calculated as the JC69 corrected distance) decreases as the average length of time that an individual spends in the seed bank increases (see Supplementary Materials). The lines in both **a)** and **b)** are the slopes from simple linear regression.


a)
Birth
Preserves genetic diversity
Resuscitation
Active
Dormant
Initiation
Death
Death
b)
Birth
Birth
Migration2,1
Population 1
Population 2
Migration1,2
Death
Death


The distribution of reductase acivity in a microbial population
1e−3
1.0
0.8
0.6
0.4
0.2
0.0
Probability
Resucitation
Dormancy
8000
7000
6000
5000
4000
3000
2000
1000
0
Reductase-associated fluorescence


a)
Nucleotide diversity (π), log_{10}
$\theta = 1.0 \cdot 10^{-3}$
$\theta = 1.0 \cdot 10^{-4}$
$\theta = 1.0 \cdot 10^{-5}$
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
Avg. time in seed bank (generations), log_{10}
b)
Nucleotide diversity (π)
Time in seed bank = 10
Time in seed bank = 100
Time in seed bank = 1000
0.05
0.04
0.03
0.02
0.01
0.00
0
2000
4000
6000
8000
10000
Time (generations)


a)
Frequency of favored allele
1.0
0.8
0.6
0.4
0.2
0.0
Time in seed bank = 10000
Time in seed bank = 1000
Time in seed bank = 100
10^0
10^1
10^2
10^3
10^4
Time (generations), log_{10}
b)
T_{fix}
10^5
10^4
10^3
10^2
10^1
Active and dormant
Active
Dormant
10^0
10^1
10^2
10^3
10^4
Average time in seed bank, log_{10}


a)
Evolutionary distance
(root-to-tip distance)
Non-spore forming
Spore forming
Number of sporulation genes, log_{10}
b)
Evolutionary distance
(JC69-corrected distance)
Average time in seed bank (generations), log_{10}