Seminars in Cell & Developmental Biology 88 (2019) 46–53

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Links between evolutionary processes and phenotypic robustness in microbes

Jeremy Draghi

Department of Biology, Brooklyn College, The Graduate Center, City University of New York, United States

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form 16 February 2018
Accepted 15 May 2018
Available online 19 June 2018

Keywords:
Stochasticity
Bet-hedging
Robustness
Evolvability
Microbial evolution

ABSTRACT

The costs and benefits of random phenotypic heterogeneity in microbes have been vigorously debated and experimental tested for decades; yet, this conversation is largely independent from discussion of phenotypic robustness in other disciplines. In this review I connect microbial examples of stochasticity with studies on the ecological and population-genetic consequences of phenotypic variability. These topics illustrate the complexity of selection pressures on phenotypic robustness and provide inspiration that this complexity can be parsed with theoretical advances and the experimental power of microbial systems.

Contents

1. Introduction

A primary value of studying phenotypic robustness is the attention it requires to the question of whether variability is good or bad. More precisely, for which traits, ecological contexts, and scales does natural selection favor organisms that are less than perfectly robust in expressing their genotypes as phenotypes? The scope of this question hints at the scale of the challenge: phenotypes, environments, and the developmental processes that connect genotypes and traits are each incredibly diverse. Microbial studies have blazed trails through this tangled wilderness, linking molecular sources of variability to distributions of phenotypes in populations and fitnesses of genotypes over evolutionary time. Yet these few pioneering examples are the exception, and recent advances in the study of stochastic phenotypic variation in microbes have developed largely independently of the conversation about robustness in the evolutionary literature.

This review attempts to connect subfields by discussing how phenotypic variability is acted upon by natural selection and, in turn, mediates the action of natural selection on new mutants. In part, I want to draw attention to a subtle aspect of the relationship between robustness and evolvability: phenotypic variability shapes the strength of selection and genetic drift, with genome-wide consequences for the fates of beneficial and deleterious mutations. While genetic drift lies at the heart of our modern understanding of genome evolution, its effects and consequences have rarely been directly measured by evolutionary biologists. High-throughput characterization of variation in phenotypes and in reproductive success can therefore shed light on a key mechanism

E-mail address: Jeremy.Draghi21@brooklyn.cuny.edu

https://doi.org/10.1016/j.semcdb.2018.05.017
1084-9521/

of evolution while also stimulating demand for a more fine-grained theory of how evolution acts on variability.

Rather than attempt an exhaustive review of either the theoretical or experimental literature surrounding these sizeable questions, my goal is to assemble a toolkit which both evolutionary and systems biologists could use to approach questions about phenotypic variability in a population context. My examples will focus on microbes to reflect the tremendous increase in capacity to measure their individual phenotypes as well as the widespread enthusiasm for adaptive explanations for the evident lack of robustness in key phenotypes like growth rate. The fact that genetic drift in microbes does not match the assumptions of the basic Wright-Fisher model has been noted [1] but often ignored in practice, further motivating the development of tools suitable for unraveling the outcomes of evolution experiments in bacteria, viruses, and unicellular eukaryotes.

2. A brief overview of stochastic phenotypic heterogeneity in microbes

Bacterial motility is a canonical example of the predominant and unintuitive role of stochasticity at the scale of single cells. Every aspect of swimming via flagella is an adaptation to the effects of Brownian motion, which prevents cells from making steady progress up or down concentration gradients [2]. Both single-cell measurements and first-principles modeling have shown that stochasticity plays a similarly important role in gene expression. The origins of much of this stochasticity in molecular diffusion, collisions, and birth-death events makes it sensible to refer to it as 'noise.' The low copy number of key proteins and transcripts within a cell amplifies this noise [107,3]. Modeling transcription, translation and decay as Poisson processes predicts substantial noise that is only compounded by stochasticity in partitioning during cell division [4] and, in eukaryotes, bursts of transcription driven by changes in chromatin structure [5,6].

Recognition of the scope and importance of expression noise in microbes has been fueled by technical advances; now-classic experiments using microscopy with pairs of fluorescent-protein reporter genes with identical promoters were instrumental in separating the noise intrinsic to gene expression from other sources of heterogeneity among cells [7–9]. Recent advances in isolating single cells and assaying their gene expression have helped accelerate these studies [10], as have developments in the mathematical modeling of gene expression. While these new techniques produce eye-catching, data-rich results, appreciation for stochasticity in microbial phenotypes actually has a long history. The classic *lac* operon requires stochastic bursts of permease production in order to allow lactose into the cell, stimulating full expression of the operon [11]. Genetic competence and sporulation are two other canonical examples of phenotypic heterogeneity in bacteria microbiology that are driven by stochasticity in gene expression. Genetic competence—the ability to ingest and homologously recombine environmental DNA—is typically expressed in only a fraction of a clonal bacterial population. A number of studies in the gram-positive bacterium *Bacillus subtilis* have shown how gene expression noise and positive feedback cycles can account for this heterogeneity, allowing testable predictions of mutational targets that shift the fraction of competent cells [12]. *B. subtilis* has other behaviors that are expressed in response to cues, but only in a fraction of a clonal population, including the production of dormant but durable spores, biofilm formation, and a cannibalism phenotype. A combination of genetics, single-cell expression assays, and mathematical modeling has demonstrated an essential role for expression noise and additional positive feedback loops in producing these heterogeneous behaviors in specific environments [13,14].

Bacterial persister cells are a telling example of the complexity underlying the simple phenotype of apparently random growth arrest. Over 70 years ago, microbiologists observed that antibiotics typically fail to kill a small minority of sensitive cells. These survivors were not resistant—they could be cultured into antibiotic-sensitive cultures—and so were described as 'persistent' [15]. In *E. coli*, persisters were shown to be transiently non-growing cells that arose stochastically in homogenous, clonal cultures [16], though later studies have shown that persister cells are not simply dormant [17]. Screens for *E. coli* strains with a higher propensity for persister formation [18] pointed to a toxin-antitoxin system. Such systems, composed of a long-lasting toxin protein and a short-lived antitoxin gene product, were first understood as a feature deployed by plasmids to promote 'addiction'—any daughter cells that, through segregation, failed to inherit the plasmid would suffer the effects of the toxin [19]. This 'selfish' role in maintaining plasmids in bacterial populations does not account for the existence of chromosomal copies of toxin-antitoxin operons, leading to a diversity of hypotheses for the role of these *prima facie* hazardous elements [19]. Detailed characterization and modeling of high-persistence mutants have shown that interactions between chromosomally encoded toxin and antitoxin gene products create a threshold response by which fluctuations in the abundances of each molecule causes temporary growth-arrest in a fraction of a growing *E. coli* population [20]. Like competence and sporulation, the fraction of persister cells in a culture is sensitive to numerous environmental signals and is also mutable [21]; in fact, the frequency of persister cells evolves rapidly and predictably in response to regimes of antibiotic treatment [22]. The results of further mutation screens imply multiple mechanisms underlying persistence, across species and even within *E. coli* [23], suggesting more sophistication and potential for adaptive tuning of this behavior.

In addition to whole-cell phenotypes like growth rate, microbes can also vary significantly in key metabolic characteristics. Galactose signaling in yeast presents an example of a complex, looping pathway in which both positive and negative feedbacks conspire to produce expression states that can change stochastically, yet also persist over several generations of division [24]. Highly asymmetrical partitioning of cellular resources has been described in *Sinorhizobium melilotias* a potential adaptation to starvation [25]. Similarly, stochastic expression differences in stress-response genes allows a fraction of cells to be pre-adapted to a severe stress without the bulk of the population bearing the burden of constitutive expression [26]. Stochastic heterogeneity in metabolism may also be relevant in an industrial context [27], and efforts to control or leverage this noise in bioprocessing applications could yield a rich source of data on the potential to evolve and engineer phenotypic robustness.

Much of the initial research on stochastic phenotypes like persistence and competence in microbes has focused on bistability—a situation in which positive feedback and threshold responses produce two quasistable phenotypes, with random variation in protein abundances mediating the transition between states [28]. These same processes can constitute a mechanism of epigenetic inheritance or cellular memory which, rather than relying on methylation or other forms of genome modification, is transmitted by the transcripts and proteins segregated during division. This type of non-genetic inheritance of phenotype may be relevant for metabolic responses to fluctuating environments [29] and can facilitate cooperation among related cells [30]. The full implications of these mechanisms of inheritance of acquired characteristics for robustness and evolution await much more modeling and characterization.

More detail on the mechanisms and phenotypic consequences of noise in microbes can be found in numerous recent reviews (e.g., [31,32]). The brief survey above should serve to illustrate

the enthusiasm for measuring noise and robustness as a preamble to discussing evolutionary explanations for this noise. Two further pieces of context should be mentioned before continuing. One, interest in microbial phenotypic heterogeneity is partially driven by medical relevance—the phenomenon of persister cells by itself constitutes a major clinical concern [33]—and the many roles of stochastic variation in metazoans, from the differentiation of photoreceptor cells in the vertebrate eye [34] to the resistance of cancers to treatments [35]. Understanding these motives can help evolutionary biologists detect biases in adaptive explanations, contribute most usefully to this growing interdisciplinary field, and make the best use of these incredibly productive sources of data on robustness.

A second issue arises from the search for the proper level of description of bacteria. One viewpoint, explored in the next section, takes these stochastic differences among microbial cells as phenotypic heterogeneity among individuals. Through this lens, the action of selection on a diverse microbial population can directly inform our understanding of how evolution acts on random differences in phenotype arising from the development of multicellular organisms. An alternative viewpoint, with some support in the bacterial literature, is that stochastic differentiation among microbial cells is further evidence that these cells act, evolve, and should be thought of as multicellular units [36,37]. These ideas are provocative but may perpetuate a false dichotomy. Answering the question of whether a microbial population is truly a collection of individuals or a multicellular collective can be productively avoided by applying formal models of the evolution of social behavior [38].

3. Evolution of phenotypic robustness in static environments

The most obvious evolutionary prediction about random phenotypic heterogeneity is that it will be selected against when the environment favors a single, unchanging trait value. However, several interesting complications arise in applying this simple expectation to stochastic variation in cells. First, theoretical work has shown that negative feedback is a surprisingly poor solution to the problem of noise in gene expression [39–42]. The problem is quite fundamental. The nature of expression noise means that a protein's abundance provides only imperfect information on the activity of its gene; therefore, no feedback mechanism using information about the protein's abundance can fully control noise, and in fact poorly tuned negative feedback mechanisms make noise worse [41]. Because the activity of the gene and abundance of its mRNA changes over time, this imperfect information can also not simply be averaged over time to allow arbitrarily precise control. While negative feedback is clearly part of cellular homeostasis, these findings reinforce the idea that intuition can be a poor guide to life at scales dominated by stochasticity.

An alternative method of noise-reduction lies in adjusting the parameters of expression, preserving the mean expression level of a gene while reducing its variance. Because mRNA abundances are typically smaller than the pools of their corresponding proteins, this could be achieved by simultaneously increasing the transcription rate and decreasing the translation rate [43,12,44,45], or by other similar opposing changes in parameters like protein and transcript lifetimes or protein affinity for its ligand. Regulation in decay and translation rates does appear to be significant and evolvable [46], providing one ingredient required for this argument. However, at first glance such evolutionary steps might seem to be constrained; changes to any single parameter of expression would change the mean as well as the variance, with potentially deleterious consequences if the mean had already evolved to be optimal. Draghi and Whitlock [47] explored this question and found that gene expression could evolve lower noise without any involvement of deleterious mutations, though the process would be slow and gradual. The key insight is that the mean and variance of gene expression are positively correlated such that a slight decrease away from the optimal mean can be favored because it is accompanied by a decrease in variance. Such a change then allows for beneficial mutations that increase the mean while increases variance somewhat, resulting in a net improvement in canalization. One prediction of this work is that robustness should evolve fastest in very large populations; in contrast, models that assume that deleterious mutations must fix might predict more rapid evolution in small populations (see [48] for a full discussion of this issue).

A more fundamental complication to the prediction that high robustness is optimal in a static environment comes from a yeast experiment that finds a growth advantage specifically associated with random heterogeneity [49,50]. At least part of this advantage comes from the epigenetic inheritance of growth rate; high-fitness cells replicate more often by definition and, if they also have a tendency to transmit their high fitness, contribute disproportionately to the population's growth rate. This result was predicted by a model of 'phenotypic memory,' which encompasses inheritance of phenotype properties by methylation patterns, or the direct inheritance of cytoplasm and membrane contents found in microbes reproducing by fission [51]. While this surprising phenomenon has yet to be broadly demonstrated and may not be quantitatively significant, it should serve to demonstrate the deep difference between microbes and the types of multicellular organisms which have served as the models for the development of evolutionary theory.

4. Evolution of robustness and variability in heterogeneous environments

Fitness can be simply defined as the expected reproductive success associated with a trait or genotype in a specific environment. However, this definition elides the issue of time: over what duration is it most appropriate to measure reproductive success? Evolutionary biologists have produced concepts and quantitative tools to integrate snapshots of fitness in specific environments into long-term predictions about successfully management of the risks and opportunities posed by environmental uncertainty and change. These concepts have been tremendously influential, but significant theoretical issues and the difficulty of achieving empirical proofs of optimality justify a healthy skepticism about the state of the field.

One starting point for a long-term view of fitness is Cohen [52], who found that random delays in reproduction could improve the long-term growth of a population in environments in which the potential for reproductive success varied with time. Cohen deployed the influential metaphor of a genotype as an investor deploying a portfolio of varied investments to maximize success in an unpredictable economy. Stearns [53] traced the history of the geometric mean in both economics and evolutionary biology as a tool for determining the optimal strategy in a varying environment, ultimately pinning the origin of the idea to Daniel Bernoulli in 1738. Although there is limited evidence of cross-pollination from economic sources like Bernoulli, evolutionary biologists converged on the geometric mean fitness as the best representation of which genotypes would prosper when environments changed across time [54].

One intuition typically offered for the validity of the geometric mean as a long-term measure of evolutionary success is that organisms, like capital, can grow exponentially; the geometric mean, which takes the nth root of the product of growth rates across n generations, reflects this multiplicative nature [53]. This explanation loses a bit of the economic argument for the geometric mean

which it is worthwhile to recover. The geometric mean fitness can be well-approximated as the arithmetic mean fitness minus a term proportional to the variance in the success of a genotype over time; put simply, the geometric mean penalizes high-risk, high-reward strategies. In economics this penalty reflects risk-aversion among investors, deriving from the empirical observation that in most situations it is more painful to lose money than it is beneficial to gain the same amount (reviewed in [55]). Orr [56] presents an argument that this risk-aversion does have an analogy in population genetics: the success of a genotype is diminished more by the loss of an individual than it is improved by the gain of an individual. The implication that selection should favor individual-level robustness of reproductive success will be further discussed below.

Despite its intuitive appeal and firm mathematical foundations, the geometric mean is technically only a valid predictor in the limit as population size approaches infinity. In finite populations, strategies that perform poorly in rarely encountered environments may outcompete the purported optimum [57,58]. This issue is particularly acute when the time-scale of significant environment change is comparable to the fixation time of an allele; because fixation time grows with the logarithm of the population size, this issue may be most relevant for microbial populations. Recent progress [59] toward a fully stochastic understanding of this hard-to-model regime promises substantial revisions in the near future to the mathematics of predicting the optimal phenotype.

While the geometric mean can be applied to predict optimal phenotypes without any further nomenclature, it is very often invoked in concert with the concept of bet-hedging. 'Bet-hedging' is a confusingly general term for adaptive responses to environmental unpredictability. The root idea is that a bet-hedging strategy avoids commitment to a particular outcome of a random process; more concretely, a bet-hedger does not fully commit, developmentally, to a phenotype that would be optimal in only one of the possible future environments in which it might find itself. The confusion arises because this avoidance of commitment can plausibly take the form of a consistent, robust phenotype or a portfolio of strategies generated by a lack of robustness in certain traits.

Imagine two environments, *A* and *B*, and two phenotypes, **a** and **b**, with fitness relations such that $w_a > w_b$ in environment *A* and $w_a < w_b$ in environment *B*. A genotype that always produces **a** or always produces **b** has committed to a particular environment; if the environment varies in such a way that individuals find themselves unpredictability placed in either *A* or *B*, then these committed strategies reflect the opposite of bet-hedging. An alternative strategy would be realized by a genotype that always produces a phenotype intermediate between **a** and **b**. Labeling this phenotype **ab**, then if $w_a > w_{ab} > w_b$ in environment *A* and $w_a < w_{ab} < w_b$ in environment *B*, then this strategy may be more successful than either committed strategy, particularly if fitness is concave-down with increasing distance from the optimal phenotype. Robust production of an intermediate phenotype, when more extreme phenotypes would fare better in a subset of the encountered environments, is known as conservative bet-hedging [60]. The term 'diversifying bet-hedging' is then used to indicate a very different approach to the same end of risk management: the stochastic production of several possible phenotypes from a given genotype. A genotype which produced **a** in a fraction *p* of individuals and **b** for the remaining fraction 1-*p* would qualify as such a strategy. In eukaryotes, diversifying bet-hedging is distinguished from a third strategy called adaptive coin-flipping (e.g., [61]), in which a parent will effectively chose a phenotype from a distribution and produce a clutch of offspring, each with that same phenotype. While this distinction may be relevant for some microbes such as viruses, in unicellular systems adaptive coin-flipping has not typically been invoked.

Any of these types of bet-hedging can achieve a higher geometric fitness than more specialized phenotypes by reducing the variance across generations of the fitness of a genotype. A conservative bet-hedger achieves this reduction in variance through its defining feature of consistent, perhaps mediocre, performance across the range of likely environments. A diversifying bet-hedger achieves the same reduction in variance by reducing the correlation in performance among individuals of the same genotype in the same environment [62]. At the level of genotype fitness, these types of bet-hedging can be equivalent; so too can these strategies be mixed in a genotype that, for example, stochastically produces several variants of a generalist phenotype. Consideration of formal models of risk management can therefore help reveal the common principles underlying the misleading dichotomy between the two extremes of conservative and diversifying bet-hedging [60].

Diversifying bet-hedging is a highly influential, adaptive hypothesis for the types of phenotypic heterogeneity in microbial population documented above. Characterization of mutants with altered distributions of stochastic phenotypes has allowed experiments testing the competitive fitness of different bet-hedging strategies in varying lab environments [63]. Beaumont et al. [64] provided one of the clearest demonstrations that stochastic heterogeneity, amounting to a diversifying bet-hedging strategy, could evolve *de novo* in *Psuedomonas fluorescens* in the lab. While this experiment deployed clever, highly contrived selection and growth regimes to strongly favor stochastic diversity, in a follow-up paper Rainey et al. [65] lament the difficulties in establishing general criteria for the evolution of bet-hedging. One problem is that producing definitive evidence of bet-hedging requires assaying fitness of many phenotypes across many environments [66]. This is most tractable for microbes, but making the inference from high performance across a range of lab environments to superior performance in nature is an implausibly wide jump for most species. This leads to the additional difficulty of comparing diversifying bet-hedging to the many alternatives: conservative bet-hedging (robustness of phenotype), adaptive plasticity in response to cues, genetic differentiation, or other mechanisms of risk-management like dispersal. For example, several theoretical studies have successfully described conditions favoring the stochastic formation of dormant forms [67–70] or other specialized phenotypes [43,71–74]. While each approach has made significant progress in mapping the competitive outcomes of two or three alternative risk-mitigating strategies, no-one has fully addressed the much harder problem of identifying circumstances in which this type of diversifying bet-hedging is optimal.

While proving optimality of diversifying bet-hedging is still out of reach, inspiration can be found in some exemplary efforts to model the complexity. Svardal et al. [75] considered the joint evolution of phenotypic means and variances in a modeling approach allowing polymorphism, finding that "one type becomes a canalized specialist for the more common ecological conditions and the other type takes the role of a de-canalized bet-hedger thriving on the less-common ecological conditions." Tufto [76] ambitiously combined genetic evolution, adaptive plasticity and diversifying bet-hedging in a quantitative-genetics framework, synthesizing and broadening the results of previous models. Scheiner [77] studied the impact of genetic architecture and life cycle on the competition between plasticity and bet-hedging, using an individual-based simulation approach. Each of these papers acknowledges a debt to Bull [78], whose model of the joint evolution of genetic and phenotypic variation has been highly influential. These examples also demonstrate the diversity of theoretical approaches used to tackle this complex problem. This diversity reflects the convergence of interest in adaptive explanations for phenotypic robustness or instability, but also indicates the potential for synthetic approaches to make still more progress.

Both conservative and diversifying bet-hedging meet the criterion of avoiding commitment to any one of several possible outcomes, and both can be superior to strategies specialized to particular environments by virtue of a higher geometric mean fitness. However, there are key differences worth noting. First, my informal observation is that nearly all references to bet-hedging in the recent microbial literature are specific to diversifying bet-hedging, often implicitly. In an otherwise laudable attempt to alleviate sloppiness in the use of the term, de Jong et al. [79] perpetuates this misconception. The focus on diversifying bet-hedging is not surprising given that immense interest in making sense of the wealth of experimental evidence for phenotypic heterogeneity in microbes, and accords with the broader trend toward diversifying bet-hedging across the field [66]. Still, there is a danger of circular reasoning: if stochastic diversity is seen as the *only* way to manage unpredictable risk, then the hypothesis of diversifying bet-hedging may face diminished scrutiny and be subject to premature acceptance as an adaptive explanation for observed heterogeneity.

A second more general distinction is that we could postulate conservative and diversifying bet-hedging strategies with equal geometric mean fitnesses but differences in evolvability—specifically, the capacities of the resulting populations to fix beneficial mutations. Both strategies achieve their benefits by reducing the variation in reproductive success of their genotype. A conservative bet-hedging strategy does this by producing a middle-of-the-road phenotype which is never extremely good or bad. In contrast, a diversifying bet-hedging strategy produces individuals which still experience both highs and lows in terms of individual fitness; reduced variation in the success of the genotype comes about because these individual fates are uncorrelated [60]. As a consequence, random variation in reproductive success within a generation is higher in a genotype producing a diversity of phenotypes, which by definition increases the strength of genetic drift and therefore decreases the odds of any beneficial mutation achieving fixation. These consequences of a non-robust strategy toward individual fitness are foundational in evolutionary theory but not widely republished or accessible to the broader community. Crow and Kimura's classic textbook (1970) shows that the probability of fixation of a beneficial mutation is approximately $p_{fix} \approx 2s^{N_e}/N$ (p. 426) rather than the more-often cited $p_{fix} \approx 2s$; here N_e is the effective population size, which may differ from the census size N for several reasons, including an excess of variation in reproductive success within a generation (p. 110). These results, which are discussed in the context of haploid microbes experiencing stochastic heterogeneity by Wang and Zhang [80] and Mineta et al. [81], point to a hidden cost of managing risk through stochastic diversity rather than a single robust, if mediocre, phenotype.

This increase in genetic drift can also be compounded by a decrease in the strength of selection for mutations that affect only some parts of a bet-hedger's phenotypic repertoire. Diversifying bet-hedgers, as generalists, suffer the evolutionary cost of relaxed selection: any genes that are not expressed as selected traits in all environments will consequently experience less effective selection, both in favoring adaptive changes and in opposed deleterious mutations [82–86,108]. For example, a gene expressed only during a persister stage of a bacteria may accumulate deleterious changes without opposition during periods when dormancy is not beneficial. Any change that improves the phenotypes of persister cells, say by adjusting the period of dormancy to fit the schedule of environmental stresses, will be effectively neutral during periods of normal growth and consequently show only a small net probability of fixation. These two factors suggest that diversifying bet-hedging might, on paper, be an optimal strategy, but in practice might be unachievable or unsustainable by evolution, even in the large populations typical of microbes.

5. Robustness of individual reproductive success may be weakly selected but consequential for evolvability

One of the challenges in applying the idea of bet-hedging stems from the multiple levels at which risk can be managed in a population. The reproductive behaviors of an individual can be more or less robust in terms of the consistency of the outcome; for example, a clutch of offspring can be deposited into a single patch or spread among several patches. If the fates of offspring in different patches are independent, then the latter behavior will produce lower variation in the number of surviving offspring. This type of individual-level diversified bet-hedging is intuitively sensible to anyone with a risk-averse mindset, but is it actually favored by natural selection? Gillespie [87,88] showed that selection does favor genotypes with a less variable distribution of offspring numbers, but that the strength of this selection term was inversely proportional to the population size. Therefore, if the behaviors associated with risk-spreading impose even a very small burden on individual fitness, robustness in reproductive success will not be favored by natural selection in large populations.

The reason this benefit to robustness is so small arises from the assumption that the relevant environmental variation is sufficiently fine-grained that individuals of a common genotype will sample different environments within one generation. If the reproductive successes of individuals are uncorrelated, then the average fitness of a genotype in a generation is nearly independent of individual-level variability [60,89]. Essentially, the success of the genotype is already hedged against risk by its representation across many individuals in a large population, making further risk reduction superfluous.

While this result suggests that variability or robustness in reproductive success is effectively neutral in large populations, one complication arises out of theoretical considerations of population structure. If a species is best described as a metapopulation—a collection of semi-independent subpopulations or demes connected by migration—then, depending on the details of the life cycle, a large metapopulation can display the relatively strong selection against reproductive variability consistent with the small size of a single deme [90–92]. Models of bacteria as metapopulations are relatively few and focus largely on altruistic social interactions (e.g., [93]) or interactions across trophic levels [94], suggesting an opportunity to apply these abstract models specifically to robustness in bacteria. Other theory shows that genotypes with high variance in reproductive success outperform low-variance types with equal mean fitness at low population densities; at densities close to carrying capacity, the competitive edge switches to favor the low-variance type [95]. These theoretical results suggest that measurements and modeling of the fine-scaled ecology, demography, and population structure of microbes are essential complements to observations of the stochastic variability of growth rates.

Despite these intriguing modeling results, we cannot definitely say that robustness in reproductive success *per se* is sufficiently favorable to make an evolutionary prediction. Individual variation in reproductive success does however correlate negatively with evolvability, as described in the previous section. These facts admit a range of interesting possibilities. If selection favoring greater evolvability is sufficiently strong, that influence alone might select for phenotypic robustness. While the strength of selection favoring evolvability is outside the scope of this review, both theoretical [96] and empirical examples [97] show that traits determining evolvability can evolve by virtue of their effects on the production of beneficial variation. If, on the other hand, robustness in repro-

ductive success is unconstrained by evolvability, then microbes in particular might vary greatly in the strength of genetic drift and therefore in their capacities to fix new beneficial mutations and exclude deleterious changes.

6. Positive effects of phenotypic heterogeneity on evolvability

Balancing the negative population-genetic effects on selection and genetic drift are a host of potentially positive effects of stochastic heterogeneity on the rate or probability of adaptation. Summarizing this work is outside the scope of the review, but a few points can be touched upon. Heterogeneity can stimulate evolution if genotypes that sometimes produce an adaptive phenotype can, by a few mutations, evolve into genotypes that produce the adaptive phenotype more consistently. This idea, traceable to Waddington [98] if not earlier, has been reviewed elsewhere (e.g., [99]) and has been explored particularly fruitfully in computational models [100]. Despite decades of theoretical interest, this topic has not been synthesized with recent discoveries regarding epigenetics and stochastic variation in microbes. Heterogeneity can also act to smooth fitness landscapes, potentially transforming an impassable series of peaks and valleys into a steady uphill climb [101].

Microbial experiments are continuing to test and challenge these old ideas. A remarkable recent experimental evolution study with yeast found that phenotypic heterogeneity in the expression of a costly antifungal resistance gene enhanced adaptation and in fact evolved to increase over the course of adapting to high concentrations of the antifungal [102]. The authors suggest that stochastic heterogeneity achieved a more favorable balance between the costs and benefits of this gene, though significant questions remain. A molecular evolution study in yeast showed evidence that translation errors provided a phenotype which was later made constitutive by mutation [103], providing a compelling, mechanistic example of the ideas discussed by Waddington.

Future work can better dissect the costs and benefits of heterogeneity by taking advantage of the ability to independently vary demographic and environment aspects of microbial experiments. Batch culture, in which a large population is severely bottlenecked before reintroduction to fresh medium to achieve regular periods of exponential growth, is often chosen for its strong practical advantages. However, the demographics of this routine may affect selection on heterogeneity as well as the dynamics of beneficial mutations [104]. Experiments contrasting evolution in the more constant populations typical of chemostats with batch culture experiments can isolate the effects of demographic variability from other types of environmental change. Clever experiments using liquid-handling robotics can also simulate metapopulations, in which selection acts on both the ability to colonize virgin habits and compete in dense, established subpopulations (e.g., [93]). Theorists need to make strong eco-evolutionary arguments for the utility of exploring microbial phenotypes in complex or labor-intensive types of growth conditions to further motivate this important work.

7. Conclusions

Microbiologists study organisms with vast population sizes subject to periods of rapid growth, intense competition and massive culling. It is therefore no surprise that this community readily embraces adaptive explanations for phenotypes. The development of new technologies and statistical approaches to high-throughput phenotyping in microbes has been somewhat overshadowed by next-generation sequencing, but the patent evidence for stochastic heterogeneity has inspired new enthusiasm for evolutionary models of strategic approaches to environmental uncertainty. Without raining on this parade, I have attempted to assemble observations with models to ascertain how much of the phenotypic heterogeneity presented by microbes is really accounted for by evolutionary theory. Large portions of the territory remain unmapped. Selection for robustness of reproductive success, for example, is a niche topic within evolutionary theory that is tremendously important for quantitatively understanding adaptation in microbes. The idea that phenotypic memory through epigenetics could allow organisms to sample possible phenotypes and then, through selection, enrich the next generation for successful variants is unorthodox within evolutionary theory to say the least. Serious attention to these discoveries will reveal whether the lack of separation between somatic and germline tissues in microbes necessitates a more specific body of evolutionary theory, or whether lessons learned in microbes will prove generalizable to multicellular life. Findings that feedbacks within and between genes are inadequate to control noise, and in fact have sometimes evolved to amplify it, should have substantial implications for theories of epistasis and evolution in gene networks. Stochastic heterogeneity has clearly been leveraged by evolution to achieve striking adaptions, even as the broader claim that evolution favors heterogeneity remains controversial [105].

While much has been written about the connections between various forms of robustness and evolvability (e.g., [106]), I have tried here to highlight some connections I feel have been underappreciated. Microbial evolution experiments in the next decade or two will have a decisive impact on these debates by enabling the quantification of evolutionary rates and causes at an unprecedented scale. Still, without a matching commitment to measuring environments and demographics of microbes in nature, we will still not understand the role of robustness and heterogeneity in these microbial model workhorses species. Evolutionary biologists can best contribute by building models that demand attention on all causally important factors, not just those for which measurement has become cheap, easy, or fashionable.

Acknowledgments

Work on the review was partially supported by NSF 13-510 Systems & Synthetic Biology, award #1714550. Thanks to Lisa Bono, Monica Saw-Aung, and Abraham Alex for useful feedback.

References

[1] Z. Patwa, L.M. Wahl, The fixation probability of beneficial mutations, J. R. Soc. Interface 5 (28) (2008) 1279–1289.
[2] H.C. Berg, *E. coli* in Motion, Springer-Verlag, NY, 2004.
[3] A. Eldar, M.B. Elowitz, Functional roles for noise in genetic circuits, Nature 467 (7312) (2010) 167.
[4] D. Huh, J. Paulsson, Non-genetic heterogeneity from stochastic partitioning at cell division, Nat. Genet. 43 (2) (2011) 95–100.
[5] K. Miller-Jensen, S.S. Dey, D.V. Schaffer, A.P. Arkin, Varying virulence: epigenetic control of expression noise and disease processes, Trends Biotechnol. 29 (10) (2011) 517–525.
[6] C.R. Brown, H. Boeger, Nucleosomal promoter variation generates gene expression noise, Proc. Natl. Acad. Sci. U. S. A. 111 (50) (2014) 17893–17898.
[7] M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Stochastic gene expression in a single cell, Science 297 (2002) 1183–1186.
[8] L. Cai, N. Friedman, X.S. Xie, Stochastic protein expression in individual cells at the single molecule level, Nature 440 (2006) 358–362.
[9] J.R.S. Newman, S. Ghaemmaghami, J. Ihmels, D.K. Breslow, M. Noble, J.L. DeRisi, J.S. Weissman, Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise, Nature 441 (2006) 840–846.
[10] X. Shi, W. Gao, J. Wang, S.H. Chao, W. Zhang, D.R. Meldrum, Measuring gene expression in single bacterial cells: recent advances in methods and micro-devices, Crit. Rev. Biotechnol. 35 (4) (2015) 448–460.
[11] P.J. Choi, L. Cai, K. Frieda, X.S. Xie, A stochastic single-molecule event triggers phenotype switching of a bacterial cell, Science 322 (5900) (2008) 442–446.
[12] H. Maamar, A. Raj, D. Dubnau, Noise in gene expression determines cell fate in *Bacillus subtilis*, Science 317 (5837) (2007) 526–529.
[13] D. Lopez, H. Vlamakis, R. Kolter, Generation of multiple cell types in *Bacillus subtilis*, FEMS Microbiol. Rev. 33 (1) (2009) 152–163.

[14] A. Chastanet, D. Vitkup, G.C. Yuan, T.M. Norman, J.S. Liu, R.M. Losick, Broadly heterogeneous activation of the master regulator for sporulation in *Bacillus subtilis*, Proc. Natl. Acad. Sci. U. S. A. 107 (18) (2010) 8486–8491.
[15] O. Gefen, N.Q. Balaban, The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress, FEMS Microbiol. Rev. 33 (4) (2009) 704–717.
[16] N.Q. Balaban, J. Merrin, R. Chait, L. Kowalik, S. Leibler, Bacterial persistence as a phenotypic switch, Science 305 (5690) (2004) 1622–1625.
[17] M.A. Orman, M.P. Brynildsen, Dormancy is not necessary or sufficient for bacterial persistence, Antimicrob. Agents Chemother. 57 (7) (2013) 3230–3239.
[18] H.S. Moyed, K.P. Bertrand, *hipA*, a newly recognized gene of Escherichia coli K-12 that affects frequency of persistence after inhibition of murein synthesis, J. Bacteriol. 155 (2) (1983) 768–775.
[19] L. Van Melderen, M.S. De Bast, Bacterial toxin–antitoxin systems: more than selfish entities? PLoS Genet. 5 (3) (2009) e1000437.
[20] Eitan Rotem, Adiel Loinger, Irine Ronin, Irit Levin-Reisman, Chana Gabay, Noam Shoresh, Ofer Biham, Nathalie Q. Balaban, Regulation of phenotypic variability by a threshold-based mechanism underlies bacterial persistence, Proc. Natl. Acad. Sci. U. S. A. 107 (28) (2010) 12541–12546.
[21] E. Maisonneuve, K. Gerdes, Molecular mechanisms underlying bacterial persisters, Cell 157 (3) (2014) 539–548.
[22] B. Van den Bergh, J.E. Michiels, T. Wenseleers, E.M. Windels, P.V. Boer, D. Kestemont, L. De Meester, K.J. Verstrepen, N. Verstraeten, M. Fauvart, J. Michiels, Frequency of antibiotic application drives rapid evolutionary adaptation of Escherichia coli persistence, Nat. Microbiol. 1 (2016) 16020.
[23] R.A. Fisher, B. Gollan, S. Helaine, Persistent bacterial infections and persister cells, Nat. Rev. Microbiol. 15 (2017) 453–464.
[24] M. Acar, A. Becskei, A. van Oudenaarden, Enhancement of cellular memory by reducing stochastic transitions, Nature 435 (7039) (2005) 228–232.
[25] W.C. Ratcliff, R.F. Denison, Individual-level bet hedging in the bacterium *Sinorhizobium meliloti*, Curr. Biol. 20 (19) (2010) 1740–1744.
[26] William J. Blake, G.ábor Balázsi, Michael A. Kohanski, Farren J. Isaacs, Kevin F. Murphy, Yina Kuang, Charles R. Cantor, David R. Walt, James J. Collins, Phenotypic consequences of promoter-mediated transcriptional noise, Mol. Cell 24 (6) (2006) 853–865.
[27] F. Delvigne, Q. Zune, A.R. Lara, W. Al-Soud, S.J. Sørensen, Metabolic variability in bioprocessing: implications of microbial phenotypic heterogeneity, Trends Biotechnol. 32 (12) (2014) 608–616.
[28] J.W. Veening, W.K. Smits, O.P. Kuipers, Bistability, epigenetics, and bet-hedging in bacteria, Annu. Rev. Microbiol. 62 (2008) 193–210.
[29] G. Lambert, E. Kussell, Memory and fitness optimization of bacteria under fluctuating environments, PLoS Genet. 10 (9) (2014) e1004556.
[30] T.M. Norman, N.D. Lord, J. Paulsson, R. Losick, Memory and modularity in cell-fate decision making, Nature 503 (7477) (2013) 481–486.
[31] M. Ackermann, A functional perspective on phenotypic heterogeneity in microorganisms, Nat. Rev. Microbiol. 13 (8) (2015) 497–508.
[32] T.M. Norman, N.D. Lord, J. Paulsson, R. Losick, Stochastic switching of cell fate in microbes, Annu. Rev. Microbiol. 69 (2015) 381–403.
[33] B. Van den Bergh, M. Fauvart, J. Michiels, Formation, physiology, ecology, evolution and clinical importance of bacterial persisters, FEMS Microbiol. Rev. 41 (3) (2017) 219–251.
[34] K. Viets, K.C. Eldred, R.J. Johnston, Mechanisms of photoreceptor patterning in vertebrates and invertebrates, Trends Genet. 32 (10) (2016) 638–659.
[35] A. Pribluda, C. Cecile, E.L. Jackson, Intratumoral heterogeneity: from diversity comes resistance, Clin. Cancer Res. 21 (13) (2015) 2916–2923.
[36] J.A. Shapiro, Thinking about bacterial populations as multicellular organisms, Annu. Rev. Microbiol. 52 (1) (1998) 81–104.
[37] C. Aguilar, H. Vlamakis, R. Losick, R. Kolter, Thinking about Bacillus subtilis as a multicellular organism, Curr. Opin. Microbiol. 10 (6) (2007) 638–643.
[38] S.A. West, A.S. Griffin, A. Gardner, S.P. Diggle, Social evolution theory for microorganisms, Nat. Rev. Microbiol. 4 (8) (2006) 597–607.
[39] D.J. Stekel, D.J. Jenkins, Strong negative self-regulation of prokaryotic transcription factors increases the intrinsic noise of protein expression, BMC Syst. Biol. 2 (6) (2008), http://dx.doi.org/10.1186/1752-0509-2-6.
[40] T.T. Marquez-Lago, J. Stelling, Counter-intuitive stochastic behavior of simple gene circuits with negative feedback, Biophys. J. 98 (2010) 1742–1750.
[41] I. Lestas, G. Vinnicombe, J. Paulsson, Fundamental limits on the suppression of molecular fluctuations, Nature 467 (7312) (2010) 174.
[42] A.J. Stewart, R.M. Seymour, A. Pomiankowski, M. Reuter, Under-dominance constrains the evolution of negative autoregulation in diploids, PLoS Comput. Biol. 9 (2013) e1002992.
[43] M. Thattai, A. Van Oudenaarden, Stochastic gene expression in fluctuating environments, Genetics 167 (1) (2004) 523–530.
[44] E.M. Ozbudak, M. Thattai, I. Kurtser, A.D. Grossman, A. van Oudenaarden, Regulation of noise in the expression of a single gene, Nat. Genet. 31 (2002) 69–73.
[45] H.B. Fraser, A.E. Hirsh, G. Giaever, J. Kumm, M.B. Eisen, Noise minimization in eukaryotic gene expression, PLoS Biol. 2 (2004) e137.
[46] C. Vogel, E.M. Marcotte, Insights into the regulation of protein abundance from proteomic and transcriptomic analyses, Nat. Rev. Genet. 13 (4) (2012) 227.
[47] J. Draghi, M. Whitlock, Robustness to noise in gene expression evolves despite epistatic constraints in a model of gene networks, Evolution 69 (9) (2015) 2345–2358.
[48] D.B. Weissman, M.W. Feldman, D.S. Fisher, The rate of fitness-valley crossing in sexual populations, Genetics 186 (4) (2010) 1389–1410.
[49] B. Cerulus, A.M. New, K. Pougach, K.J. Verstrepen, Noise and epigenetic inheritance of single-cell division times influence population fitness, Curr. Biol. 26 (9) (2016) 1138–1147.
[50] S.F. Levy, Cellular heterogeneity: benefits besides bet-hedging, Curr. Biol. 26 (9) (2016) R355–R357.
[51] O. Carja, J.B. Plotkin, The evolutionary advantage of heritable phenotypic heterogeneity, Sci. Rep. 7 (2017) 5090.
[52] D. Cohen, Optimizing reproduction in a randomly varying environment, J. Theor. Biol. 12 (1) (1966) 119–129.
[53] S.C. Stearns, Daniel Bernoulli (1738): evolution and economics under risk, J. Biosci. 25 (3) (2000) 221–228.
[54] J.H. Gillespie, Natural selection with varying selection coefficients–a haploid model, Genet. Res. 21 (2) (1973) 115–120.
[55] N.C. Barberis, Thirty years of prospect theory in economics: a review and assessment, J. Econ. Perspect. 27 (1) (2013) 173–196.
[56] H.A. Orr, Absolute fitness, relative fitness, and utility, Evolution 61 (12) (2007) 2997–3000.
[57] O.D. King, J. Masel, The evolution of bet-hedging adaptations to rare scenarios, Theor. Popul. Biol. 72 (4) (2007) 560–575.
[58] C.J. Graves, D.M. Weinreich, Variability in fitness effects can preclude selection of the fittest, Annu. Rev. Ecol. Evol. Syst. 48 (2017) 399–417.
[59] I. Cvijović, B.H. Good, E.R. Jerison, M.M. Desai, Fate of a mutation in a fluctuating environment, Proc. Natl. Acad. Sci. U. S. A. 112 (36) (2015) E5021–E5028.
[60] J. Starrfelt, H. Kokko, Bet-hedging—a triple trade-off between means, variances and correlations, Biol. Rev. 87 (3) (2012) 742–755.
[61] H. Olofsson, J. Ripa, N. Jonzén, Bet-hedging as an evolutionary game: the trade-off between egg size and number, Proc. R. Soc. Lond. B: Biol. Sci. 276 (1669) (2009) 2963–2969.
[62] S.A. Frank, M. Slatkin, Evolution in a variable environment, Am. Nat. 136 (2) (1990) 244–260.
[63] M. Acar, J.T. Mettetal, A. van Oudenaarden, Stochastic switching as a survival strategy in fluctuating environments, Nat. Genet. 40 (4) (2008) 471–475.
[64] H.J. Beaumont, J. Gallie, C. Kost, G.C. Ferguson, P.B. Rainey, Experimental evolution of bet hedging, Nature 462 (7269) (2009) 90.
[65] P.B. Rainey, H.J. Beaumont, G.C. Ferguson, J. Gallie, C. Kost, E. Libby, X.X. Zhang, The evolutionary emergence of stochastic phenotype switching in bacteria, Microb. Cell Fact. 10 (1) (2011) S14.
[66] A.M. Simons, Modes of response to environmental change and the elusive empirical evidence for bet hedging, Proc. R. Soc. Lond. B: Biol. Sci. (2011), rspb20110176.
[67] E. Kussell, R. Kishony, N.Q. Balaban, S. Leibler, Bacterial persistence: a model of survival in changing environments, Genetics 169 (4) (2005) 1807–1814.
[68] E. Kussell, S. Leibler, Phenotypic diversity, population growth, and information in fluctuating environments, Science 309 (5743) (2005) 2075–2078.
[69] A. Gardner, S.A. West, A.S. Griffin, Is bacterial persistence a social trait? PLoS One 2 (8) (2007) e752.
[70] E. Rajon, S. Venner, F. Menu, Spatially heterogeneous stochasticity and the adaptive diversification of dormancy, J. Evol. Biol. 22 (10) (2009) 2094–2103.
[71] M.C. Donaldson-Matasci, M. Lachmann, C.T. Bergstrom, Phenotypic diversity as an adaptation to environmental uncertainty, Evol. Ecol. Res. 10 (4) (2008) 493–515.
[72] T. Malik, H.L. Smith, Does dormancy increase fitness of bacterial populations in time-varying environments? Bull. Math. Biol. 70 (4) (2008) 1140–1162.
[73] J. Müller, B.A. Hense, T.M. Fuchs, M. Utz, C. Pötzsche, Bet-hedging in stochastically switching environments, J. Theor. Biol. 336 (2013) 144–157.
[74] S.M. Scheiner, Bet-hedging as a complex interaction among developmental instability, environmental heterogeneity, dispersal, and life-history strategy, Ecol. Evol. 4 (4) (2014) 505–515.
[75] H. Svardal, C. Rueffler, J. Hermisson, Comparing environmental and genetic variance as adaptive response to fluctuating selection, Evolution 65 (9) (2011) 2492–2513.
[76] J. Tufto, Genetic evolution, plasticity, and bet-hedging as adaptive responses to temporally autocorrelated fluctuating selection: a quantitative genetic model, Evolution 69 (8) (2015) 2034–2049.
[77] S.M. Scheiner, The genetics of phenotypic plasticity. XIII. Interactions with developmental instability, Ecol. Evol. 4 (8) (2014) 1347–1360.
[78] J.J. Bull, Evolution of phenotypic variance, Evolution 41 (2) (1987) 303–315.
[79] I.G. de Jong, P. Haccou, O.P. Kuipers, Bet hedging or not? A guide to proper classification of microbial survival strategies, Bioessays 33 (3) (2011) 215–223.
[80] Z. Wang, J. Zhang, Impact of gene expression noise on organismal fitness and the efficacy of natural selection, Proc. Natl. Acad. Sci. U. S. A. 108 (16) (2011) E67–E76.
[81] K. Mineta, T. Matsumoto, N. Osada, H. Araki, Population genetics of non-genetic traits: evolutionary roles of stochasticity in gene expression, Gene 562 (1) (2015) 16–21.
[82] T.J. Kawecki, Accumulation of deleterious mutations and the evolutionary cost of being a generalist, Am. Nat. 144 (5) (1994) 833–838.
[83] T.J. Kawecki, N.H. Barton, J.D. Fry, Mutational collapse of fitness in marginalhabitats and the evolution of ecological specialisation, J. Evol. Biol. (3) (1997) 407–429.

[84] R.D. Holt, Demographic constraints in evolution: towards unifying the evolutionary theories of senescence and niche conservatism, Evol. Ecol. 10 (1) (1996) 1–11.
[85] Whitlock, The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth, Am. Nat. (1996) S65–S77.
[86] E.C. Snell-Rood, J.D. Van Dyken, T. Cruickshank, M.J. Wade, A.P. Moczek, Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity, Bioessays 32 (1) (2010) 71–81.
[87] J.H. Gillespie, Natural selection for within-generation variance in offspring number, Genetics 76 (3) (1974) 601–606.
[88] J.H. Gillespie, Natural selection for variances in offspring numbers: a new evolutionary principle, Am. Nat. 111 (981) (1977) 1010–1014.
[89] K.R. Hopper, J.A. Rosenheim, T. Prout, S.J. Oppenheim, Within-generation bet hedging: a seductive explanation? Oikos 101 (1) (2003) 219–222.
[90] M. Shpak, Evolution of variance in offspring number: the effects of population size and migration, Theory Biosci. 124 (1) (2005) 65–85.
[91] L. Lehmann, F. Balloux, Natural selection on fecundity variance in subdivided populations: kin selection meets bet hedging, Genetics 176 (1) (2007) 361–377.
[92] M. Shpak, S.R. Proulx, The role of life cycle and migration in selection for variance in offspring number, Bull. Math. Biol. 69 (3) (2007) 837–860.
[93] J.R. Nahum, B.N. Harding, B. Kerr, Evolution of restraint in a structured rock–paper–scissors community, Proc. Natl. Acad. Sci. U. S. A. 108 (Supplement 2) (2011) 10831–10838.
[94] F.J. Hol, O. Rotem, E. Jurkevitch, C. Dekker, D.A. Koster, Bacterial predator–prey dynamics in microscale patchy landscapes, Proc. R. Soc. B 283 (2016) 1824.
[95] T.L. Parsons, C. Quince, J.B. Plotkin, Some consequences of demographic stochasticity in population genetics, Genetics 185 (4) (2010) 1345–1354.
[96] J. Draghi, G.P. Wagner, Evolution of evolvability in a developmental model, Evolution 62 (2) (2008) 301–315.
[97] R.J. Woods, J.E. Barrick, T.F. Cooper, U. Shrestha, M.R. Kauth, R.E. Lenski, Second-order selection for evolvability in a large Escherichia coli population, Science 331 (6023) (2011) 1433–1436.
[98] C.H. Waddington, Genetic assimilation of an acquired character, Evolution 7 (2) (1953) 118–126.
[99] M.J. West-Eberhard, Developmental Plasticity and Evolution, Oxford University Press, 2003.
[100] L.W. Ancel, W. Fontana, Plasticity, evolvability, and modularity in RNA, J. Exp. Zool. 288 (3) (2000) 242–283.
[101] S.A. Frank, Natural selection. II. Developmental variability and evolutionary rate, J. Evol. Biol. 24 (11) (2011) 2310–2320.
[102] Z. Bódi, Z. Farkas, D. Nevozhay, D. Kalapis, V. Lázár, B. Csörgő, Á. Nyerges, B. Szamecz, G. Fekete, B. Papp, H. Araújo, Phenotypic heterogeneity promotes adaptive evolution, PLoS Biol. 15 (5) (2017) e2000644.
[103] H. Yanagida, A. Gispan, N. Kadouri, S. Rozen, M. Sharon, N. Barkai, D.S. Tawfik, The evolutionary potential of phenotypic mutations, PLoS Genet. 11 (8) (2015) e1005445.
[104] L.M. Wahl, P.J. Gerrish, I. Saika-Voivod, Evaluating the impact of population bottlenecks in experimental evolution, Genetics 162 (2) (2002) 961–971.
[105] D.S. Tawfik, Messy biology and the origins of evolutionary innovations, Nat. Chem. Biol. 6 (10) (2010) 692–696.
[106] A. Wagner, Robustness and Evolvability in Living Systems, Princeton University Press, 2005.
[107] J.M. Raser, E.K. O'shea, Noise in gene expression: origins, consequences, and control, Science 309 (5743) (2005) 2010–2013.
[108] J.D. Fry, The evolution of host specialization: are trade-offs overrated? Am. Nat. 148 (1996) S84–S107.