Bet hedging or not? A guide to proper classification of microbial survival strategies

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Bacteria have developed an impressive ability to survive and propagate in highly diverse and changing environments by evolving phenotypic heterogeneity. Phenotypic heterogeneity ensures that a subpopulation is well prepared for environmental changes. The expression bet hedging is commonly (but often incorrectly) used by molecular biologists to describe any observed phenotypic heterogeneity. In evolutionary biology, however, bet hedging denotes a risk-spreading strategy displayed by isogenic populations that evolved in unpredictably changing environments. Opposed to other survival strategies, bet hedging evolves because the selection environment changes and favours different phenotypes at different times. Consequently, in bet hedging populations all phenotypes perform differently well at any time, depending on the selection pressures present. Moreover, bet hedging is the only strategy in which temporal variance of offspring numbers per individual is minimized. Our paper aims to provide a guide for the correct use of the term bet hedging in molecular biology.

Keywords:
bacteria; bet hedging; evolution; evolutionarily stable strategy; phenotypic heterogeneity

Introduction

In their natural environment, microorganisms are usually subject to frequent and largely unpredictable changes. Isogenic individuals of microbial populations often respond with the development and the production of offspring with diverse phenotypes [1] to increase the chance of survival of at least a subpopulation in altered environmental situations [2, 3]. This phenotypic heterogeneity can be generated by intra- and/or extracellular noise, e.g. stochastic variations in gene expression or differences in local concentrations of extracellular nutrients [4, 5]. Nutrient concentrations can be sensed and processed by intracellular genetic networks. If the corresponding network topology is characterized by being bi- or multistable, it can generate phenotypic heterogeneity (for a recent review see ref. 3). Recently, several cases of observed phenotypic heterogeneity in bacterial populations have been suggested to be 'bet hedging' [3, 6–19]. Apparently, bet hedging is used by molecular and cell biologists to describe the observation that microorganisms potentially increase the continuity of their DNA by displaying different phenotypes within one population. However, there are several types of phenotypic heterogeneity, with different characteristics, that have developed due to different evolutionary causes. Calling all such phenomena 'bet hedging' will lead to misunderstandings between evolutionary and molecular biologists, and may lead to wrong conclusions. The aim of our essay is to stimulate the correct use of the term bet hedging in molecular and cell biology. As a first step towards the correct identification of survival strategies such as bet hedging, our essay focuses on introducing non-

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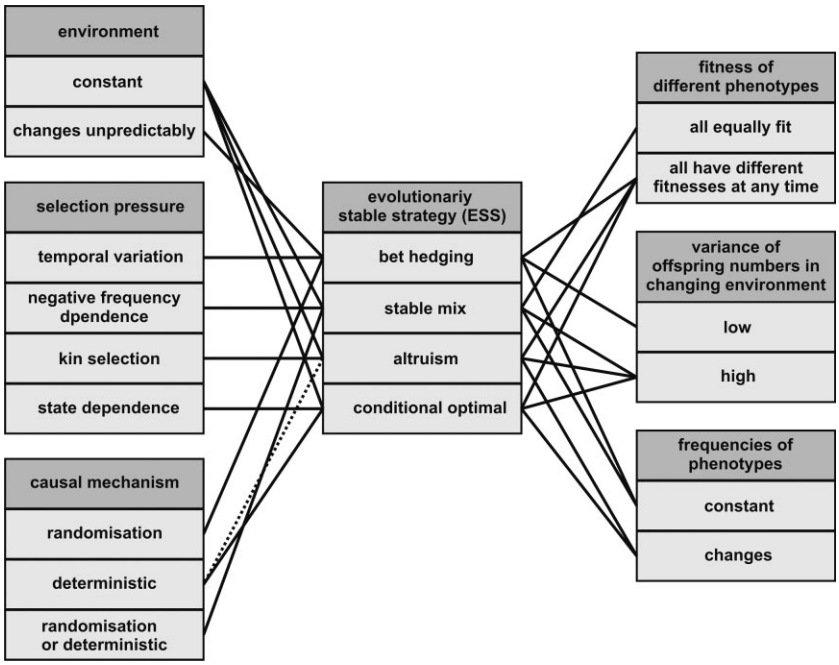

Figure 1. Overview of evolutionarily stable strategies displaying phenotypic heterogeneity and their corresponding causes and characteristics. Continuous line: known connection; dotted line: expected connection.

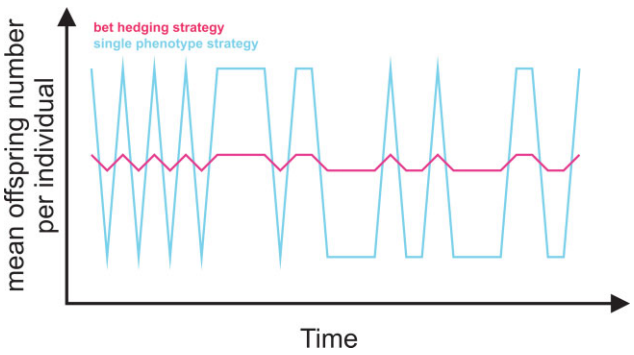

Figure 2. Illustration of the reduction in variance exhibited by a bet hedging population (red) compared to a homogeneous phenotype strategy displayed by another population (blue). The population with only one phenotype performs well in some environments and badly in others, whereas each phenotype of the bet hedging strategy performs differently well in different environments. The result is a minimized variance in offspring number of a bet hedging population compared to a single phenotype population.

evolutionary biologists, like molecular and cell microbiologists, to the concept of bet hedging and its evolutionary context.

What is bet hedging?

The concept bet hedging refers to survival strategies that have evolved due to specific environmental conditions and which display distinct characteristics (Fig. 1): bet hedging is a risk-spreading strategy displayed by isogenic populations that evolved in unpredictably changing environments. One of its characteristics is that temporal variance of offspring numbers per individual is minimized (Fig. 2). Bet hedging theory was developed in the 1970's as part of evolutionary theory [20, 21]. Evolutionary theory is based on various conceptual models which are used to examine connections between ultimate causes (selection pressures) and the characteristics of observed traits (evolutionary outcome). These models reflect reality in a strongly simplified way, in order to be able to single out the effects of specific ultimate causes without the confounding effects of additional factors. Hence, evolutionary biologists derive possible evolutionary outcomes for a population within a given environment by applying models which consider a limited set of features present in reality. In the particular case of bet hedging, evolutionary models are used to connect an unpredictable environment to phenotypic heterogeneity in isogenic populations (Fig. 1). In our opinion, the expression bet hedging should be reserved for risk-spreading strategies of isogenic, not polygenic populations, since the models that were used to develop the concept assumed that genotypes are fixed, apart from the occurrence of rare, spontaneous mutations [20, 22].

In order to avoid further misconceptions, it is of utmost importance to become aware that a) the original concept of bet hedging only applies to risk-spreading strategies observed in isogenic, not polygenic populations b) that bet hedging refers to a very specific type of phenotypic heterogeneity, that evolves in unpredictably changing environments c) that other types of selection environments may also lead to phenotypic heterogeneity, which cannot be called bet hedging and d) that due to lack of information regarding the specific characteristics of the observed phenotypic heterogeneity and/or the preceding selection environment many known cases of phenotypic heterogeneity observed in isogenic bacterial populations can currently not be identified as bet hedging with absolute certainty (see below).

Overall, it appears that there is a great need for clarification of evolutionary terminology for the application in molecular and cellular microbiology (Box 1). To understand the concept bet hedging properly and to place it in perspective to other heterogeneous strategies, it is important to understand the underlying conceptual approach as well as related evolutionary concepts and terms, which are described below.

Evolutionary concepts and expressions related to bet hedging

Selection, resident population and evolutionarily stable strategy

Evolutionary theory states that environmental conditions select for advantageous genotypes (not individuals!). Hence,

Box 1

Altruists
Individuals that invest in a process of which the outcome is advantageous for other individuals and costly to themselves

Bet hedging
Risk-spreading strategy based on phenotypic heterogeneity which provides a long-term fitness advantage in uncertain time-varying environments compared to other strategies

Evolutionarily stable strategy (ESS)
A strategy that cannot be invaded by any initially rare mutant which is similar to its ancestors

Inclusive fitness
Fitness gain of individuals as a result of helping relatives to survive and/or propagate

Isogenic population
Population in which all individuals are genetically identical

Kin selection
Selection pressure which is defined by the characteristic that altruistic individuals increase their inclusive fitness

Negative frequency dependence
Selection pressure which is defined by the characteristic that the most common phenotypes have decreased fitness compared to rare phenotypes

Phenotype
Summary of traits displayed by one individual

Phenotypic heterogeneity
Observation describing that an isogenic or a polygenic population consists of more than one phenotype

Phenotypic plasticity
Ability to alter the phenotype in response to the experienced environment

Polygenic population
A population that consists of individuals which share some, but not all genetic information

Resident population
Currently established population

Selection
Process whereby the relative frequency of individuals with favoured traits increases

Selection pressure
Evolutionary factor that favours specific traits. Individuals that display favoured traits have increased fitness, while others that do not exhibit the traits have decreased fitness

Temporal variation
Selection pressure that is not constant

Trait
Characteristic that affects survival and/or reproductive success

traits that affect survival and/or reproduction of an organism are subject to selection, and mutations repeatedly generate new genotypes, which may or may not have selective advantages. Genotypes coding for traits that have a selective advantage will in the long run outcompete other, less favoured genotypes. When the corresponding population lives under given environmental conditions for a very long period, the surviving individuals will be maximally adapted to those conditions. Note, that environmental conditions refer to either constant selection pressures, or selection pressures that change in a predictable or unpredictable manner.

As discussed above, theoretical models are used to study the outcome of evolutionary processes. These models share some features (like mutation and selection), but specific details of those features vary between models, depending on the evolutionary process that is studied. Since the concept of bet hedging and other evolutionary strategies are based on these models, we describe the basic features of the most common type of models (Fig. 3): an evolutionary process is assumed to start with a population consisting of only one genotype. This isogenic population is named the 'resident population'. Mutations can occur, which generate initially rare genotypes. In most cases it is assumed that mutations are rare and that they have small effects, i.e. the mutant individuals resemble their ancestors. The occurrence of new mutants can result in two possible outcomes: the mutant subpopulation can either grow (invade the resident population) or die out. In the latter case, the resident population is restored until a new mutation arises. In case of successful invasion, the mutants will in the long run either co-exist with their ancestors or outcompete them. Both scenarios result in a new resident population, which consists of either one or multiple genotypes, i.e. the corresponding resident population is isogenic or polygenic. This sequence of events is repeated until new mutants cannot invade the resident population. The resulting stable, final state of the population is called an evolutionarily stable state [23]. The corresponding phenotype(s) of the population are defined as an evolutionarily stable strategy (ESS, Fig. 3) [24]. The characteristics of an evolutionarily stable strategy are determined by the selection pressures under which it has evolved (Fig. 4). Currently known types of selection

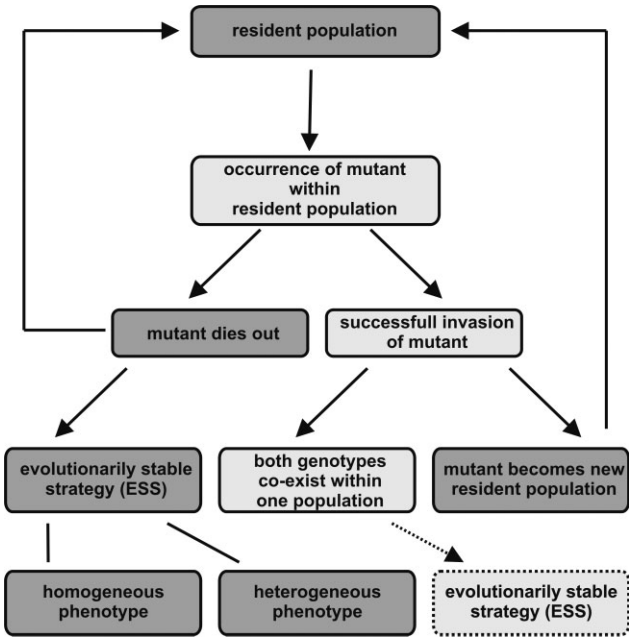

Figure 3. Basic features of common models applied to investigate evolutionary processes in theory. An isogenic population (dark grey) is considered as the first population of a theoretical experiment. This population is termed the 'resident' population. Mutations occur, resulting in a population of different genotypes (light grey). The corresponding mutants either die out, upon which the resident population is re-established, or they successfully invade the population. In the latter case, different genotypes either co-exist, or the mutants become the new resident population. If an isogenic population cannot be invaded by any nearby mutant (nearby = very similar to its ancestor), then the population displays an evolutionarily stable strategy, which can either consist of one or of several phenotypes. Similarly, the dotted lines indicate that polygenic populations can also evolve to evolutionarily stable states, but we only focus on isogenic populations here.

pressures that may give rise to an evolutionarily stable strategy with phenotypic heterogeneity in isogenic populations are: temporal variation [25], negative frequency dependence [26], kin selection [27] and state dependence [28]. In the following section, these types of selection pressures and their consequences are discussed in order to clarify why phenotypic heterogeneity described by bet hedging is

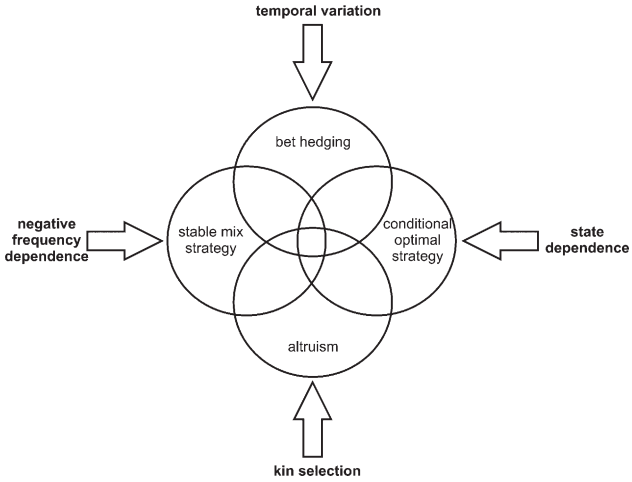

Figure 4. Illustration of some theoretically possible combinations of evolutionarily stable strategies and the corresponding selection pressures.

distinct from other phenotypic heterogeneity phenomena (see also Table 1 and Fig. 1).

Evolutionary causes of phenotypic heterogeneity in isogenic populations

Temporal variation

In many cases selection pressures vary over time, so that different phenotypes perform well in different periods. For instance, the local availability of nutrients for soil bacteria is usually not constant, but changes as the result of events like the death of an earthworm. Therefore, soil bacteria experience long periods with low resources that are interrupted by sudden availability of nutrients. Such unpredictably changing selection environments can result in several evolutionary outcomes, depending on the magnitude and frequency of the environmental changes (e.g. [29]). If the changes are great enough, and occur frequently, then phenotypic heterogeneity may evolve within isogenic populations. This conclusion was first derived by Gillespie in 1973 [20, 25]. A surprising finding of his study was that such a strategy

Table 1. Characteristics of different evolutionarily stable strategies

	Causal mechanism	Reaction to environmental change	Individual reproduction and survival (fitness)	Reaction to change in population composition	Per-capita reproduction in a changing environment
Evolutionarily stable strategy	**Randomization**	**Population composition changes**	**Different for different phenotypes**	**Reproductive success of different phenotypes changes**	**Variance in time**
Bet hedging	Yes	No	Yes	No	Low
Stable mix strategy	Yes/no	No	No	Yes	High
Altruism	Yes/no	Yes	Yes	Yes	High
Conditional optimal strategy	No	Yes	Yes	Yes/no	High

can outcompete alternative strategies which have higher average offspring numbers (Fig. 2). This is due to the fact that phenotypic heterogeneity reduces the variance in offspring numbers over time, which provides a competitive advantage in changing environments [20, 25, 30, 31]. Phenotypic heterogeneity in this case involves risk-spreading since it ensures that in any of the environments that are encountered at least a subset of the population can survive and reproduce. The expression 'bet hedging' was introduced by Slatkin because of this aspect [21].

It is important to realize that in the case of bet hedging, individuals with different phenotypes have different survival chances and reproductive success relative to each other, and furthermore, that these fitnesses change in time depending on the selection pressure present. Characteristics of the selection environment, such as the magnitude and frequency of changes, determine the mean, the variance as well as other distributional characteristics of the phenotype distribution [32, 33]. For instance, isogenic *B. subtilis* MB24 cells have been shown to sporulate with an efficiency of about 15% in a non-controlled batch culture [34]. The observed sporulation efficiency might be the result of a selection process that favours this efficiency not only over a sporulation efficiency of 0 or 100% (non-sporulating vs. non-reproducing population), but also over an isogenic population with a sporulation efficiency of 20%.

Negative frequency dependence

Negative frequency dependent selection occurs in situations where rare phenotypes have an advantage over common ones [26]. It is clear that under such circumstances different phenotypes will be maintained in the population. There is no commonly used expression for the resulting evolutionarily stable strategy. We will refer to it as 'stable mix' strategy. Negative frequency dependence probably plays a role in the maintenance of host immune type variance. Pathogens usually adapt rapidly to the most common host immune types. This gives individuals with rare immune types a selective advantage. A similar argument accounts for the maintenance of heterogeneity in pathogen phenotypes. The majority of hosts are adjusted to the most common pathogen, giving a fitness advantage to rare types of pathogens [35]. As a consequence, the interplay between hosts and pathogens promotes heterogeneity in both and ultimately, the host as well as the pathogen population will contain a stable distribution of phenotypes – hence, stable mix strategy. Opposed to bet hedging, every individual is equally fit within this evolutionarily stable strategy distribution, regardless of its phenotype [24].

Kin selection

Kin selection occurs in situations where individuals promote the propagation of their genes by increasing the reproductive success of their close relatives (their so-called 'inclusive fitness'), even if the consequence is reduction of their own direct fitness [1, 36]. In this case, the evolutionarily stable strategy contains altruistic individuals and selfish individuals which profit from the altruists. An example is bacterial protease production. It has been observed that only part of an isogenic bacterial population invests energy to produce proteases required for degradation of compounds in the environment prior to utilization of the nutrients [37]. Importantly, these nutrients not only benefit the protease producing individuals, but all individuals of the population. Protease-producing individuals are altruists, since they have lower survival and offspring numbers compared to the selfish individuals that can invest more energy in growth and reproduction. Nevertheless, the propagation of the genes of the altruists is promoted by the higher reproductive success of non-altruists, since all individuals in the population are genetically identical. Notably, the fitness of non-altruists is higher in populations with altruists than in populations without altruists [36].

State dependence

State dependent selection occurs if differences in individual history or local circumstances lead to variations in individual states within an isogenic population (e.g. [38, 39]) and if different phenotypes are optimal for different individual states. In such a situation, the evolutionarily stable strategy is a so-called 'conditional optimal strategy' [28]. For instance, Garcia and colleagues [38] showed that heat resistance of spores produced by *B. weihenstephanensis* KBAB4 depends on the temperature at which sporulation is induced. This is a conditional optimal strategy if induction temperature is a good predictor of future temperature, and if heat resistance is costly.

Combinations

The different types of selection pressures that may result in phenotypic heterogeneity within isogenic populations can also occur simultaneously (Fig. 4). For instance, the timing of initiation of sporulation varies between individuals but might nevertheless be adjusted in response to the level of nutrients or ambient temperature (cf. [7, 40]). This may be explained by simultaneous occurrence of temporal variation and state dependent selection. Accordingly, sporulation heterogeneity may be called 'conditional bet hedging'. In this case the phenotype distribution depends on the environmental signal that partly predicts the next selection environment [32, 41].

Identifying bet hedging

In most cases, populations are subject to a combination of different selection pressures (Fig. 4). This implies that bet hedging as the sole explanation for phenotypic heterogeneity is very rare, if it occurs at all. Nevertheless, bet hedging is likely to play a role in many observed cases of phenotypic heterogeneity. In the following, we explain how bet hedging can be identified in theory (see also Table 1 and Fig. 1) and why the expression bet hedging is unlikely to be correctly applied to many cases of phenotypic heterogeneity displayed by bacteria. Furthermore, we provide suggestions for future research that potentially

helps to place survival strategies displayed by bacteria in an evolutionary context.

The difficulty of identifying bacterial bet hedging in practice

Issues that appear when studying evolutionary processes in bacteria arise from the fact that the conceptual models that underlie bet hedging as well as the other three strategies discussed here were developed with multicellular organisms like plants and insects in mind. They were applied, e.g. to explain heterogeneity in timing of seed germination, or diapause times. Furthermore, during the time of development, mechanisms like epigenetic inheritance were not (yet) generally accepted. Most models assumed that the phenotype is determined by birth and to be fixed after that, although some models were used to consider phenotypes that are able to respond to environmental conditions during early development [42]. Molecular biological research has uncovered that microbial genomes as well as phenotypes may change rapidly in response to selection environments and the number of molecular origins of phenotypic variation has increased. Accordingly, the genetic composition of bacterial populations is one factor needing attention, when investigating the possible role of bet hedging in bacteria.

It is generally accepted that bacterial populations are isogenic provided they originate from one common ancestor, e.g. after repeated re-streaking of a colony to single colonies. Of course the presumed clonal reproduction is accompanied by mutations, especially when the bacteria are grown under non-optimal growth conditions. In theory, it is easy to establish whether bacteria are (still) isogenic after several generations. The genomes are relatively small and can be sequenced within acceptable time periods. Practically, it is not feasible to check the genetic content of a (let alone every) bacterial population of interest, since depending on the duration of the experiment high numbers of cells will be present, which might mask rare mutations. Furthermore, the costs and time required for rigorous sequencing would considerably expand, resulting in research delays. Thus, strictly spoken, bet hedging cannot be unequivocally determined to play a role in bacterial evolution, unless it is proven that the population of interest is indeed isogenic. However, it is commonly accepted in molecular microbiology to compromise on this point. In reality, the conceptual models underlying evolutionarily stable strategies need further development to allow the application for bacteria (e.g. [43–45]).

Molecular and cell biology are disciplines that definitely can provide new inputs for future research in evolutionary biology. The understanding of the cause and outcome of an evolutionary process should promote communication between molecular biologists and evolutionary biologists as well as joined research efforts regarding selection pressures, molecular mechanisms and their evolutionary outcomes.

Research directions that may contribute to the identification and understanding of bacterial evolutionarily stable strategies

Information about the selection environment in which an evolutionarily stable strategy evolved can help to determine whether bet hedging plays a role (Figs. 1 and 4, Table 1). However, in many cases, phenotypic heterogeneity is observed rather than obtained by evolutionary experiments in which selection pressures are known or controlled. If information on the selection environment is not available, several empirical strategies can be used to distinguish bet hedging from other evolutionarily stable strategies. The different types of evolutionarily stable strategies (bet hedging, stable mix strategy, altruism, conditional optimal strategy) that evolve due to the four different types of selection pressures (temporal variation, negative frequency dependence, kin selection, state dependence) have in common that they imply phenotypic heterogeneity within isogenic populations. However, each strategy has different characteristics (Table 1, Fig. 1).

In the following, research lines are proposed, that can help to increase the understanding of evolutionary processes in bacteria. The information may in turn be used by evolutionary biologists to develop new, bacteria-specific conceptual models or to expand existing models, so that evolutionarily stable strategies evolved in bacterial populations can be classified correctly.

Fitness-related research

Risk-spreading is only involved in environments that change unpredictably; in this case a genotype 'hedges its bets' with different phenotypes. The next experienced environment may then turn out to be good for some of the phenotypes, whereas others may perish. Such an element of risk–spreading in which every phenotype is differently fit, does not occur in any of the other strategies (Table 1, Fig. 1). Thus, possible research strategies concern the study of individual fitness components, like survival and reproduction. Time-lapse fluorescence microscopy is a relatively new technique that can be applied to investigate survival and reproduction of potential bet hedging populations. If the gene for a fluorescent protein (e.g. GFP or mCherry) is fused to a promoter which is specific for a trait of interest, then time-lapse fluorescence microscopy can be used to analyse and compare fitness-related behaviour of single cells. An additional advantage is that the history of single cells can be followed at the end of an experiment, and that different traits can be visualized simultaneously by combining different fluorescent proteins [7, 14].

If it turns out that the fitness of selected phenotypes is equal, then the population displays the stable mix strategy. Furthermore, altruists do not have a higher fitness than non-altruists under any circumstances; and the fitness of phenotypes of conditional optimal strategies is deterministically determined by an individual's state.

Similar experiments can be designed which examine how fitness components change under different circumstances. The same type of experiments will provide information on variations of phenotype distributions after environmental changes. In the case of bet hedging, this should not affect the distribution of phenotypes, whereas in most of the other cases it will. For the stable mix strategy, we expect that it will induce an initial change, after which the population will return to the stable strategy distribution. The phenotype

distributions of conditional optimal strategies and altruism will change (see Table 1). One experiment we envisage is to study the reproductive success of the population in a changing environment. Time-lapse microscopy could therefore be performed in a so-called flow chamber which allows the change of medium compositions (e.g. [46]). In the case of a bet hedging strategy, the average numbers of offspring per individual should have a low variance in time. In the case of a stable mix strategy this depends on whether or not the population composition is affected by the experiment. For the other types of evolutionarily stable strategies we expect a high fitness variance. Another possible experiment is to select individuals of different phenotypes from their population and introduce them in a population with a different phenotypic composition. One way to isolate subpopulations with a specific trait is fluorescence-activated cell sorting (FACS), which separates fluorescently labelled cells of a mixed population. The subpopulation of interest can be mixed with another population and its fitness can be analyzed by (time-lapse) microscopy or flow cytometry. In the case of a bet hedging strategy, the transfer of a bed hedging subpopulation to another population should not affect the fitness of the selected phenotypes, as long as other environmental conditions are kept constant. In the case of a conditional strategy, there should also be no change, as long as the individual's state is not affected by the experiment. Phenotypes from a stable mix strategy should encounter changes in fitness, since the new population composition is different. The same goes for altruism, since the fitness of altruists also depends on the number of individuals within the population.

Research concerning proximate causes of phenotypic heterogeneity

The advantage of studying bacteria is that the direct molecular mechanisms that generate phenotypic heterogeneity may be observed to some extent. Bet hedging strategies can only be generated well by randomization (e.g. [47]), whereas stable mix strategies can be either randomized or deterministically determined and conditional optimal strategies are generated deterministically. Altruistic behaviour could be determined randomly, but it is more likely that it is generated deterministically, as well, since the proximity of relatives plays a large role here. The single cell techniques described above already have been applied to study direct molecular causes that generate phenotypic heterogeneity (e.g. [7, 14]).

Excluding bet hedging

In cases when it is difficult or seemingly impossible to analyze whether observed phenotypic heterogeneity is (partially) due to bet hedging, it may at least be possible to determine whether bet hedging can be excluded as the underlying strategy (also see Fig. 1 and Table 1). One possible experiment would be to artificially increase the number of cells which display a specific trait (e.g. [48]). If the reduced phenotypic heterogeneity results in an increased mean fitness as well as a decrease in the variation of fitness, then the original population did not display bet hedging concerning that trait.

General research lines

Other research lines that may increase the understanding of evolutionary processes in general would be to elucidate and characterize the evolutionary changes that occurred between currently available laboratory strains and their original free-living ancestors, i.e. the original isolates that were taken from the environment. It is commonly known that strains used by different laboratories exhibit different phenotypes and genotypes compared to each other. It would be interesting to characterize these differences and to elucidate why some show more similarity to the original resident strain than others. Will the original strain or currently used laboratory strains adapt faster or better to new selection environments? Which type is more prone to invasion? Can a laboratory strain re-evolve to its ancestor? Research related to these types of questions will greatly improve our understanding of evolutionary processes in more detail. And the knowledge can in turn be implemented in models predicting the behaviour of bacteria in response to given or predicted environmental changes. The information could be conferred onto relevant clinical pathogens, as well as on the effect of environmental changes on bacteria in nature.

An initial step towards understanding the evolution of bet hedging has recently been achieved by Beaumont et al. [11].They managed to empirically select for a bet hedging bacterial genotype by performing a series of selection experiments in which the bacterium *Pseudomonas fluorescens* was subjected to alternating cultivation in a stirred or stagnant medium. Subsequent to each round of cultivation, mutants with different phenotypes from the ancestor were isolated and cultivated further according to the same regime. After several selection rounds Beaumont et al. found a mutant which produced a random mixture of two different phenotypes. Apparently, a single gene determined this phenotypic heterogeneity, which was able to evolve only after eight preceding mutations in other genes. This study clearly demonstrates that bet hedging can evolve in time varying selection environments. The chosen selection regime, however, is probably not so common, since it involves the repeated occurrence of severe bottlenecks in combination with selection for rarity. On the other hand, such a selection regime might occur in pathogens, where successful infection of hosts occurs by one or few individuals that succeed in overcoming the initial immune response of the host. In accordance with this idea, such immune responses usually favour rare types [35].

Summarized, information on the selection environment preceding phenotypic heterogeneity in isogenic populations as well as on the fitness of different phenotypes, on the changes in phenotype distributions after environmental change, and on the direct molecular mechanisms causing phenotypic heterogeneity, can be used to identify the type of evolutionarily stable strategy. On the other hand, bet hedging as well as the other strategies discussed in this paper can strictly spoken not be applied to describe phenotypic heterogeneity in bacteria, since the underlying conceptual models were developed for multicellular organisms that are isogenic and not likely to change their genome and phenotypes within short periods of time.

Conclusions and prospects

The evolutionary expression 'bet hedging' has recently been introduced in microbiological studies, but was not always applied correctly. According to the original, evolutionary context, bet hedging is a theoretical concept that describes a very specific type of phenotypic heterogeneity observed in isogenic populations. Bet hedging refers to a risk-spreading strategy that evolved in environments that changed unpredictably in time. One of its characteristics is that the variance of average offspring numbers is minimized. Correct categorization of bacterial phenotypic heterogeneity is hampered by the apparent difficulties caused by differences in terminology and concepts between different research disciplines, such as evolutionary biology and molecular microbiology and the requirement of integration of available molecular information in evolutionary concepts.

Our paper has the intention to stimulate the correct use of the expression 'bet hedging' by introducing non-evolutionary biologists like molecular and cell biologists to evolutionary definitions and mechanisms related to bet hedging. Furthermore, the corresponding information communicated in this essay should promote the interaction and collaboration of molecular and cell biologists with evolutionary biologists. By providing a common language basis we encourage researchers from different disciplines to design and execute experiments which are aimed at investigating the evolutionary and molecular origins of phenotypic heterogeneity in a common research effort. The resulting information can be used to either expand existing models in order to allow the usage of, e.g. the expression bet hedging for bacterial populations, or to develop new conceptual models that specifically consider characteristics of bacterial geno- and phenotypes.

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References

1. **Shapiro JA.** 1998. Thinking about bacterial populations as multicellular organisms. *Ann Rev Microbiol* **52**: 81–104.
2. **Dubnau D**, **Losick R.** 2006. Bistability in bacteria. *Mol Microbiol* **61**: 564–72.
3. **Veening JW**, **Smits WK**, **Kuipers OP.** 2008. Bistability, epigenetics, and bet-hedging in bacteria. *Ann Rev Microbiol* **62**: 193–210.
4. **Elowitz MB**, **Levine AJ**, **Siggia ED**, **Swain PS.** 2002. Stochastic gene expression in a single cell. *Science* **297**: 1183–6.
5. **Fraser D**, **Kaern M.** 2009. A chance at survival: gene expression noise and phenotypic diversification strategies. *Mol Microbiol* **71**: 1333–40.
6. **Piggot P.** 2010. Epigenetic switching: bacteria hedge bets about staying or moving. *Curr Biol* **20**: R480–2.
7. **de Jong IG**, **Veening JW**, **Kuipers OP.** 2010. Heterochronic phosphorelay gene expression as a source of heterogeneity in *Bacillus subtilis* spore formation. *J Bacteriol* **192**: 2053–67.
8. **Janulczyk R**, **Masignani V**, **Maione D**, **Tettelin H**, et al. 2010. Simple sequence repeats and genome plasticity in *Streptococcus agalactiae*. *J Bacteriol* **192**: 3990–4000.
9. **Jones SE**, **Lennon JT.** 2010. Dormancy contributes to the maintenance of microbial diversity. *Proc Natl Acad Sci USA* **107**: 5881–6.
10. **Fenchel T.** 2010. The life history of *Flabellula baltica Smirnov* (*Gymnamoebae, Rhizopoda*): adaptations to a spatially and temporally heterogeneous environment. *Protist* **161**: 279–87.
11. **Beaumont HJE**, **Gallie J**, **Kost C**, **Ferguson GC**, et al. 2009. Experimental evolution of bet hedging. *Nature* **462**: 90–3.
12. **Salathe M**, **van Cleve J**, **Feldman MW.** 2009. Evolution of stochastic switching rates in asymmetric fitness landscapes. *Genetics* **182**: 1159–64.
13. **Ito Y**, **Toyota H**, **Kaneko K**, **Yomo T.** 2009. How selection affects phenotypic fluctuation. *Mol Syst Biol* **5**: 264.
14. **Veening JW**, **Stewart EJ**, **Berngruber TW**, **Taddei F**, et al. 2008. Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc Natl Acad Sci USA* **105**: 4393–8.
15. **Grant AJ**, **Sheppard M**, **Deardon R**, **Brown SP**, et al. 2008. Caspase-3-dependent phagocyte death during systemic *Salmonella enterica serovar Typhimurium* infection of mice. *Immunology* **125**: 28–37.
16. **Gardner A**, **West SA**, **Griffin AS.** 2007. Is bacterial persistence a social trait? *PLoS One* **2**: e752.
17. **Rocha EP**, **Matic I**, **Taddei F.** 2002. Over-representation of repeats in stress response genes: a strategy to increase versatility under stressful conditions? *Nucleic Acids Res* **30**: 1886–94.
18. **Ratcliff WC**, **Denison RF.** 2010. Individual-level bet hedging in the Bacterium *Sinorhizobium meliloti*. *Curr Biol* **20**: 1740–4.
19. **Zhang XX**, **Rainey PB.** 2010. Bet hedging in the underworld. *Genome Biol* **11**: 137.
20. **Gillespie J.** 1974. Natural selection for within-generation variance in offspring numbers. *Genetics* **76**: 601–6.
21. **Slatkin M.** 1974. Hedging one's evolutionary bets. *Nature* **250**: 704–5.
22. **Maynard Smith J.** 1982. The century since Darwin. *Nature* **296**: 599–601.
23. **Maynard Smith J.** 1982. *Evolution and the Theory of Games*. Cambridge, UK: Cambridge University Press.
24. **Maynard Smith J.** 1974. The theory of games and the evolution of animal conflicts. *J Theor Biol* **47**: 209–21.
25. **Gillespie J.** 1973. Natural selection with varying selection coefficients – a haploid model. *Genet Res* **21**: 115–20.
26. **Allen JA**, **Clarke BC.** 1984. Frequency dependent selection: homage to E.B. Poulton. *Biol J Linn Soc* **23**: 15–8.
27. **Hamilton WD.** 1963. The Evolution of Altruistic behavior. *Am Nat* **97**: 354.
28. **McNamara JM**, **Houston AI.** 1996. State-dependent life histories. *Nature* **380**: 215–21.
29. **Meyers LA**, **Bull JJ.** 2002. Fighting change with change: adaptive variation in an uncertain world. *Trends Ecol Evol* **17**: 551–7.
30. **Seger J**, **Brockman H.** 1987. What is bet-hedging?. In Harvey P, Partridge L, Eds; *Oxford Surveys in Evolutionary Biology*. Oxford, UK: Oxford University Press. p. 182–211.
31. **Philippi T**, **Seger J.** 1989. Hedging one's evolutionary bets, revisited. *Trends Ecol Evol* **4**: 41–4.
32. **Haccou P**, **Iwasa Y.** 1995. Optimal mixed strategies in stochastic environments. *Theor Popul Biol* **47**: 212–43.
33. **Donaldson-Matasci M**, **Lachmann M**, **Bergstrom C.** 2008. Phenotypic diversity as an adaptation to environmental uncertainty. *Evol Ecol Res* **10**: 493–515.
34. **Monteiro SM**, **Clemente JJ**, **Henriques AO**, **Gomes RJ**, et al. 2005. A procedure for high-yield spore production by *Bacillus subtilis*. *Biotechnol Progr* **21**: 1026–31.
35. **Frank SA**, **Bush RM.** 2007. Barriers to antigenic escape by pathogens: trade-off between reproductive rate and antigenic mutability. *BMC Evol Biol* **7**: 229.
36. **Hamilton W.** 1964. The genetical evolution of social behaviour I and II. *J Theor Biol* **7**: 1–16 and 17–52.
37. **Veening JW**, **Igoshin OA**, **Eijlander RT**, **Nijland R**, et al. 2008. Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Mol Syst Biol* **4**: 184.
38. **Garcia D**, **van der Voort M**, **Abee T.** 2010. Comparative analysis of *Bacillus weihenstephanensis* KBAB4 spores obtained at different temperatures. *Int J Food Microbiol* **140**: 146–53.

39. **Wolf DM**, **Fontaine-Bodin L**, **Bischofs I**, **Price G**, et al. 2008. Memory in microbes: quantifying history-dependent behavior in a bacterium. *PLoS One* **3**: e1700.
40. **Chastanet A**, **Vitkup D**, **Yuan GC**, **Norman TM**, et al. 2010. Broadly heterogeneous activation of the master regulator for sporulation in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **107**: 8486–91.
41. **Iwasa Y**, **Haccou P.** 1994. ESS emergence pattern of male butterflies in stochastic environments. *Evol Ecol* **8**: 503–23.
42. **Gravilets S**, **Scheiner S.** 1993. The genetics of phenotypic plasticity. 5. Evolution of reaction norm shape. *J Evol Biol* **6**: 31–48.
43. **Wolf DM**, **Vazirani V**, **Arkin A.** 2005. Diversity in times of adversity: probabilistic strategies in microbioal survival games. *J Theo Biol* **234**: 227–253.
44. **Kussell E**, **Leibler S.** 2005. Phenotypic diversity, population growth, and information in fluctuating environments. *Science* **309**: 2075–8.
45. **Wolf DM**, **Arkin AP.** 2003. Motifs, modules and games in bacteria. *Curr Opin Microbiol* **6**: 125–34.
46. **Ducret A**, **Maisonneuve E**, **Notareschi P**, **Grossi A**, et al. 2009. A microscope automated fluidic system to study bacterial processes in real time. *PLoS One* **4**: e7282.
47. **Leimar O.** 2005. The evolution of phenotypic polymorphism: randomized strategies versus evolutionary branching. *Am Nat* **165**: 669–81.
48. **Fujita M**, **Losick R.** 2005. Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. *Genes Dev* **19**: 2236–44.