Beyond the (geometric) mean: stochastic models undermine deterministic predictions of bet hedger evolution

Maya Weissman[1,*]
Yevgeniy Raynes[1]
Daniel Weinreich[1]

1. Brown University, Providence, Rhode Island, 02912;
∗ Corresponding author; e-mail: maya_weissman@brown.edu.

Manuscript elements: Box 1, figure 1, figure 2, figure 3, figure 4. All figures to print in color.

Keywords: Bet hedging, population genetics, stochastic modeling, fluctuating environment, lineage selection, modifier theory

Manuscript type: Article.

Abstract

Bet hedging is a ubiquitous strategy for risk reduction in the face of unpredictable environmental change where a lineage lowers its variance in fitness across environments at the expense of also lowering its arithmetic mean fitness. Previously, deterministic research has quantified this trade-off using geometric mean fitness (GMF), and has found that bet hedging is expected to evolve if and only if it has a higher GMF than the wild-type. We introduce a novel stochastic framework that leverages both individual-based simulations and Markov chain numerics to capture the effects of stochasticity in the phenotypic distribution of diversified bet hedger offspring, in environmental regime, and in reproductive output. We find that modeling stochasticity can alter the sign of selection for the bet hedger compared to the deterministic predictions. We show that stochasticity in phenotype and in environment drive the sign of selection to differ from the deterministic prediction in opposing ways: phenotypic stochasticity causes bet hedging to be less beneficial than predicted, while environmental stochasticity causes bet hedging to be more beneficial than predicted. We conclude that existing, deterministic methods may not be sufficient to predict when bet hedging traits are adaptive.

Introduction

When environmental change is unpredictable, the most optimal adaptation can be difficult to predict. Is it better for a lineage to adopt a high risk and high reward strategy by producing offspring that maximize reproductive success in some environments but fail in others, to play it safe by producing offspring with a phenotype that can survive and reproduce in any environment, or to spread risk by producing phenotypically diverse offspring? Due to the multiplicative nature of reproduction, the occasional bad environment can have an out-sized effect on the long-term success of a lineage (Box 1) (Gillespie 1974, Slatkin 1974, Philippi and Seger 1989, Philippi 1993). As such, adaptations that reduce risk are especially important.

Bet hedging is a ubiquitous strategy for risk reduction in varying environments wherein a lineage sacrifices its short term fitness in order to insulate itself against uncertain environmental conditions (Slatkin 1974, Philippi and Seger 1989, Simons 2011). Mathematically, bet hedging is defined as any strategy that lowers fitness variance at the expense of mean fitness (Box 1) (Philippi and Seger 1989). More specifically, bet hedging is thought to maximize geometric mean fitness (GMF) at the expense of arithmetic mean fitness (AMF) (Philippi and Seger 1989, Philippi 1993). Other possible risk reduction strategies include adaptive tracking and phenotypic plasticity (Simons 2009, Simons 2011). Adaptive tracking is the evolution of optimal trait values via standing genetic variation and new mutations, and is thought to be most adaptive when the timescale of environmental change is very slow (Simons 2011). Phenotypic plasticity is a strategy where individuals can change their phenotype based on environmental cues, and is thought to be most adaptive when environmental change is predictable (Schlichting and Pigliucci 1998, Simons 2011). In contrast, bet hedging is thought to be most effective at reducing risk when environmental change is rapid and unpredictable (Simons 2011, Botero et al. 2015).

Bet hedging has evolved in over 29 classes across 16 phyla (Simons 2011). One well-known example of bet hedging is bacterial persistence, a strategy that allows bacteria to survive antibiotic pressure by producing a subpopulation of persister cells (Balaban et al. 2004). Persister cells divide more slowly, thereby reducing the population's mean fitness, but are able to survive longer periods of antibiotic pressure, increasing their fitness in the face of antibiotics and thus lowering fitness variance across the two environmental conditions (Balaban et al. 2019, de Jong

et al. 2011). Another canonical example is delayed seed germination in annual plants. Variable seed germination time decreases the average number of seeds that germinate each year, but also lowers the variance in fitness over time by maintaining a seed bank (Philippi 1993, Venable 2007, Childs et al. 2010). Other examples include variable egg hatching in crustaceans (Pinceel et al. 2021), mouthbrooding in cichlids (Weller et al. 2022), altruism in birds (Capilla-Lasheras et al. 2021), amphicarpic seed production in annual plants (Gianella et al. 2021), and diauxic shift in yeast (Bagamery et al. 2020).

Box 1: Defining types of means and variances. Different kinds of both mean and variance are important in understanding bet hedging. When the fitness of an allele is not constant over time, determining its long-term evolutionary outcome requires consideration of average fitness. Average fitness over time can be calculated either using the arithmetic mean (AMF) or the geometric mean (GMF). AMF is computed as the sum of a series, divided by the number of elements in the series (n). GMF, on the other hand, is computed as the nth root of the product of the series (McAlister and Galton 1997). GMF is often used in predicting long term evolutionary success because it better captures the multiplicative nature of reproduction, and therefore the importance of extremely low fitness values and temporal fitness variance (Philippi and Seger 1989). To visualize the utility of GMF, imagine a series of fitness values that includes a zero at one generation. Biologically, we know that if a lineage has a fitness of zero in one generation, it will go extinct. The arithmetic mean of a series that includes a zero will be low, but not zero. However the geometric mean of this series will be zero, better reflecting the eventual fate of the lineage (Graves and Weinreich 2017, Simons 2009).

Variance can either be measured within a generation (i.e. spatial variance) or between generations (i.e. temporal variance). While the effects of both within- and between- generation variance on bet hedging have been studied (Hopper et al. 2003, most frequently, the benefit of bet hedging is thought of as specifically reducing temporal variance (Slatkin 1974, Venable 2007). This is because temporal fitness variance will have the largest impact on multiplicative reproduction, and therefore, on the long term success of a lineage (Simons 2009, (Starrfelt and Kokko 2012). However, within-generation fitness variance still plays a role in bet hedger evolution, specifically for diversified bet hedgers, which will by definition increase their within-generation fitness variance through the production of multiple phenotypes. While we will discuss both within- and between- generation fitness variance, throughout this paper, we focus specifically on environments that only vary in time, as spatially varying environments are beyond the scope of this paper.

All bet hedging strategies can be classified into one of two categories, conservative bet hedging and diversified bet hedging, depending on the mechanism by which they reduce risk and the degree of phenotypic variation among offspring (Slatkin 1974, Philippi and Seger 1989, Childs et al. 2010, Simons 2011). To illustrate how the two different types of bet hedging strategies reduce both mean and temporal variance in fitness, consider a simplified environment that changes un-

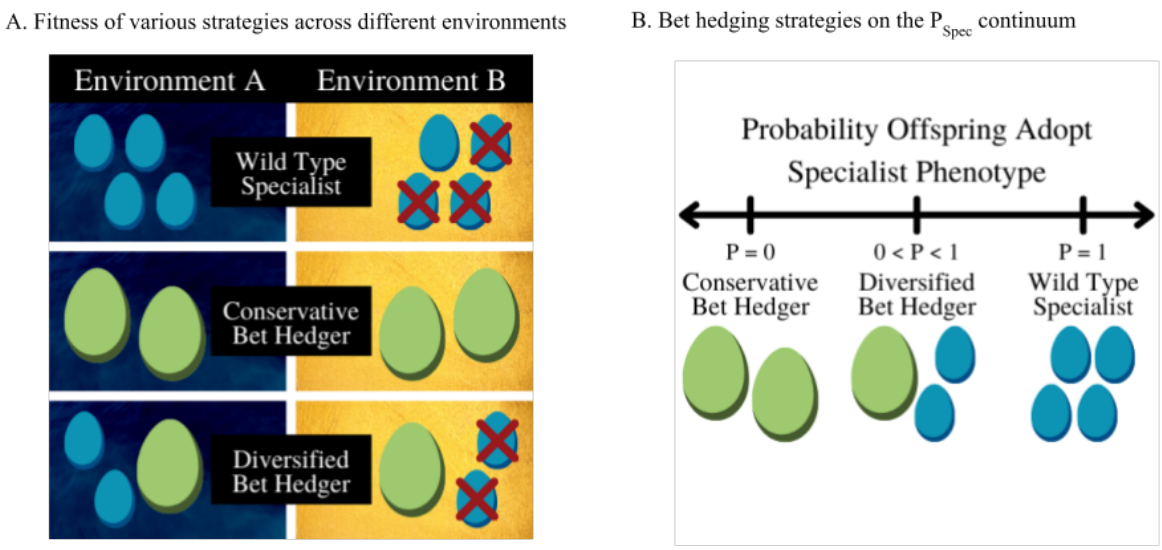

Figure 1: The benefit of bet hedging is seen through the fitness effects of different phenotypes across environmental conditions. A. The environment randomly oscillates between two states: A (stress-free) and B (high-stress). The wild-type specialist has a high fitness in Environment A and a low fitness in Environment B, resulting in both a high AMF and a high fitness variance. The conservative bet hedger produces offspring that all adopt the generalist phenotype, which has an intermediate fitness across both environments. The diversified bet hedger produces phenotypically variable offspring, such that some proportion of offspring are well adapted to the environment. B. All three strategies can exist on a continuum of the proportion of offspring that adopt the specialist phenotype as opposed to the generalist phenotype, or P_{Spec}. For a wild-type lineage, P_{Spec} = 1. A conservative bet hedging lineage has a P_{Spec} = 0. By definition, the diversified bet hedger has 0 ¡ P_{Spec} ¡ 1, where P_{Spec} proportion of offspring will adopt the specialist phenotype and $1 - P_{Spec}$ proportion of offspring will adopt the generalist phenotype.

predictably between a stress-free, nutrient rich state (Environment A) and a high-stress, nutrient-poor state (Environment B) (fig 1A). By definition, the wild-type expresses a specialist phenotype, which has a high fitness in the stress-free environment, but a low fitness in the high-stress environment. As a result, the wild-type specialist has a high temporal fitness variance (Box 1). The conservative bet hedger reduces variance by having all offspring adopt the same generalist phenotype, which has an intermediate fitness regardless of environment. For the conservative bet hedger, all offspring are "jacks of all trades, masters of none." This means that the conservative phenotype has a lower fitness than the wild-type specialist in the stress-free environment, but a higher fitness than the wild-type in the high-stress environment, resulting in a lower AMF, but potentially higher GMF, than the wild-type. Conservative bet hedging strategies lower both the within- and between-generation fitness variances (Philippi and Seger 1989, Simons 2011, Starrfelt and Kokko 2012). On the other hand, the diversified bet hedger reduces risk by producing a range of phenotypically distinct offspring, such that some offspring are better suited to each possible environment. In this way, diversified bet hedgers embody the adage "don't put all of your eggs in one basket." Like the conservative bet hedger, the diversified bet hedger has a lower fitness than the wild-type in the stress-free environment, as some proportion of offspring are maladapted, and a higher fitness than the wild-type in the high-stress environment, as some proportion of offspring are well adapted. Thus, the diversified bet hedger also has a lower AMF, but potentially higher GMF, than the wild-type. Unlike the conservative bet hedger, the diversified bet hedger has a higher within-generation fitness variance. However, because some proportion of offspring are always better adapted to any environment, the between-generation fitness variance of the diversified bet hedger is lowered, thereby reducing risk (Philippi and Seger 1989, Simons 2011, Starrfelt and Kokko 2012).

Diversified and conservative bet hedging strategies are sometimes thought of as distinct solutions, yet these two strategies can also be regarded as existing on a continuum of possible distributions of phenotypes among offspring (Olofsson et al. 2009, Venable 1985, Starrfelt and Kokko 2012). Returning to our previous scenario, the conservative phenotype is one of the phenotypes the diversified bet hedger can adopt, rather than a distinct "Environment B adapted" phenotype (fig 1B). This framing mirrors many real-world examples of bet hedging strategies. For example, in bacterial persistence, both conservative bet hedging strains of "tolerant" bacte-

ria, which all adopt the same tolerant phenotype, and diversified bet hedging "persister" strains, which exhibit phenotypic switching between the wild-type and tolerant phenotype, have been observed (Balaban et al. 2019). Additionally, within-clutch variance in egg size in salmon aligns with this continuum, as salmon can lay both small, specialist eggs and large, stress tolerant eggs in the same clutch (Einum and Fleming 2004). However, not all putative bet hedging traits align with this continuum; for some diversified bet hedgers there is no associated conservative strategy possible. Diversified bet hedgers are often classified by whether they express a specialist phenotype and a generalist phenotype (high-risk/low-risk), or two specialized phenotypes for opposing environments(high-risk/high-risk) (Venable 1985). For an example of a high-risk/high-risk diversified strategy, consider delayed seed germination in annual plants, as there is no assured "generalist" year in which the environment is guaranteed to be good. While not all bet hedgers fit the high-risk/low-risk pattern, this framework allows us to draw more direct comparisons when modeling both conservative and diversified bet hedging strategies.

Previously, the evolution of bet hedging has largely been explored in deterministic models through the lens of GMF (Gillespie 1974, Frank and Slatkin 1990, Starrfelt and Kokko 2012, Einum and Fleming 2004). In this deterministic framing, bet hedging has been thought to be beneficial only when the bet hedger has a greater GMF than the specialist wild-type (Childs et al. 2010, Simons 2011). The deterministic GMF reflects the predictions for reproductive outcome, phenotypic distribution, and environmental regime as population size and time to fixation / loss approach infinity. However, in reality, population size and time to fixation / loss are finite, which means that populations will likely experience a high degree of variance in realized reproduction, phenotype, and environment due to stochasticity. This variance in realized phenotypic distribution and in the environmental regime can have profound impacts on the evolution of bet hedging.

While a few evolutionary models of bet hedging have incorporated stochasticity in phenotype or environmental regime (Olofsson et al. 2009, Libby and Ratcliff 2019, Simons 2009, Haaland et al. 2019, Yasui 2022, Martín et al. 2019), the interaction between stochasticity in reproduction, realized phenotypic distribution in diversified bet hedger offspring, and in environment has been understudied. Here, we develop a novel stochastic framework that leverages both individual-based simulations and Markov chain numerics to simultaneously capture the effects

of stochasticity in the phenotypic distribution of diversified bet hedger offspring, in environmental regime, and in reproductive output.

We find that modeling stochasticity can alter the sign of selection for the bet hedger when the GMF of the bet hedger is greater than, equal to, and less than the wild-type. Interestingly, we find that not only can the sign of selection differ from the prediction, but that the sign of selection depends on population size. More specifically, we find that bet hedging can be favored at large population sizes but disfavored at small population sizes. This non-monotonic dependence of the sign of selection on population size is known as sign inversion (Raynes et al. 2018, Raynes et al. 2021), and means that bet hedging can be less beneficial than expected at small population size, and more beneficial than expected at large population sizes. We further show that stochasticity in phenotypic distribution and in environment drive the sign of selection to differ from the deterministic prediction. We find that across a wide range of parameter space, sign inversion occurs for both conservative and diversified bet hedgers. We conclude that the extent to which bet hedging is adaptive may differ from previous predictions, and that incorporating stochasticity is vital to adequately predict the evolution of bet hedging. As an existing issue in the field is determining when putative bet hedging strategies are adaptive, we propose that using GMF alone may not accurately predict when a trait is beneficial.

Results

Stochasticity changes the extent to which bet hedging is beneficial

Under a purely deterministic population genetics model, beneficial traits will always reach fixation and deleterious traits will always be lost. In this framing, whether or not the bet hedger is adaptive, and therefore can displace the specialist wild-type population, is determined by the GMF of both strategies: the strategy with the higher GMF will always reach fixation (Philippi and Seger 1989). In this way, GMF is often considered to be synonymous with the deterministic prediction. However in a stochastic model, we instead consider the probability of fixation for an invading mutant to assay its sign of selection. Following others (Raynes et al. 2018), we normalize the probability of fixation by the neutral expectation (classically equal to its starting frequency, 1/N), such that the neutral benchmark is always equal to 1 regardless of population

size (Raynes et al. 2018). Therefore, we can characterize the sign of selection for a trait by its normalized probability of fixation ((NP_{fix}): a beneficial trait has a probability of fixation greater than the neutral expectation ($NP_{fix} > 1$), while a deleterious trait has a probability of fixation less than the neutral expectation ($NP_{fix} < 1$) (Raynes et al. 2018).

We introduce a probabilistic model that incorporates three sources of stochasticity: stochasticity in reproduction, in phenotypic distribution, and in environment. While the expectations of these stochastic distributions (or their means) are expected to align with analogous predictions from deterministic methods, by modeling stochasticity we can capture the effect of variance in these three sources of stochasticity, as well as mean, on bet hedger evolution. Classical population genetics theory has established the role of stochasticity in reproduction, called genetic drift. As population size increases, the effect of genetic drift decreases and the efficiency of selection increases (Kimura 1962). As population size decreases and approaches 1, drift overwhelms selection and NP_{fix} converges with 1. Our other two sources of stochasticity, in phenotype and in environment, are specific to the evolution of bet hedging. Stochasticity in the environment is randomness in the sequence of environments that a bet hedging lineage experiences before reaching fixation or loss. On the other hand, stochasticity in phenotype is randomness in the distribution of phenotypes amongst diversified bet hedger offspring in a generation. By definition, a conservative bet hedger does not experience any stochasticity in phenotype, as all conservative offspring will adopt the same, low risk phenotype.

We evaluate the sign of selection for a single invading bet hedger mutant in a population of $N - 1$ wild-type specialist individuals using stochastic, Wright-Fisher simulations. The probability of fixation is calculated as the proportion of realizations in which the bet hedger mutant reaches fixation. We first consider a diversified bet hedging lineage ($P_{Spec} = 0.5$) invading a population of wild-type individuals ($P_{Spec} = 1.0$) with a GMF of 1 (fig 2). The fitness of the bet hedger is characterized by ΔGMF, or the GMF of the bet hedger subtracted by the GMF of the wild-type. Therefore, when $\Delta GMF > 0$, the bet hedger has a greater GMF and is considered beneficial in the deterministic framing. Representative values of ΔGMF are selected to show the probability of fixation when bet hedging is expected to be strongly beneficial (ΔGMF = 0.1), weakly beneficial (ΔGMF = 0.01), neutral (ΔGMF = 0), weakly deleterious (ΔGMF = -0.015), and strongly deleterious (ΔGMF = -0.1). We utilize both stochastic individual-based simulations

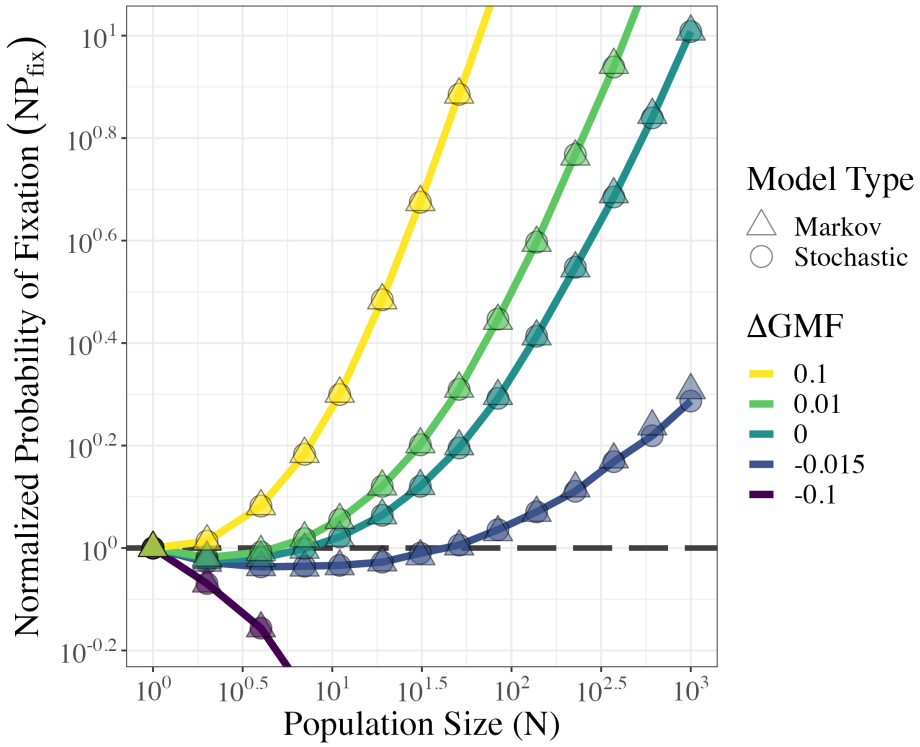

Figure 2: The normalized probability of fixation (NP_{Fix}) for a single invading diversified bet hedging mutant ($P_{Spec} = 0.5$) in a population of $N - 1$ wild-type individuals ($P_{Spec} = 1.0$) is shown as a function of population size and ΔGMF. We find no significant difference between the stochastic, individual-based simulations and Markov matrix numerics ($p > 0.99$). We compare the normalized probability of fixation to that of the neutral benchmark ($NP_{fix} = 1$) to characterize the sign of selection. When ΔGMF = 0.1 and -0.1, our results agree with the deterministic prediction that bet hedging is beneficial ($NP_{fix} > 1$) or deleterious ($NP_{fix} < 1$) across all population sizes. For the three intermediate values of ΔGMF (0.01, 0, and -0.015), we find that the sign of selection disagrees with deterministic predictions. Instead, bet hedging is deleterious at small population sizes (NPfix¡1) and beneficial at larger population sizes (NPfix¿1). It thus exhibits sign inversion.

and Markov matrix numerics, and find no difference between the two model types in results ($p = 0.9964$).

When bet hedging is expected to be strongly beneficial (here, $\Delta GMF = +0.1$), bet hedging is unconditionally beneficial ($NP_{fix} > 1$ for all $N > 1$), which aligns with the deterministic prediction. Similarly, when bet hedging is expected to be strongly deleterious (here $\Delta GMF = -0.1$), the sign of selection also agrees with the deterministic prediction and bet hedging is deleterious across all population sizes ($NP_{fix} < 1$ for all $N > 1$). Consistent with existing theory, for both above values of ΔGMF, we see that as population size decreases, $NPfix$ approaches the neutral benchmark. In other words, population size changes the efficiency of selection, but not the sign.

When the GMF of the bet hedger is equal to that of the resident wild-type ($\Delta GMF = 0$), bet hedging should be neutral according to the deterministic prediction. Instead, we find that the normalized probability of fixation is below the neutral expectation at small population sizes, crosses the neutral expectation at some population size ($N \approx 10$), and then becomes greater than the neutral expectation at larger population sizes. More simply, when $\Delta GMF = 0$, bet hedging is deleterious at small population sizes and beneficial at larger population sizes. This is notable because across almost all population sizes, the sign of selection for the bet hedger disagrees with the deterministic prediction of selective neutrality. This nonmonotonic dependence of the sign of selection on population size is known as sign inversion (Raynes et al. 2018). At large population sizes, bet hedging is more beneficial than the deterministic prediction predicts, while at small population sizes bet hedging is more deleterious than the deterministic prediction predicts. We also find that when the bet hedger is weakly beneficial or deleterious ($\Delta GMF = +0.01$ and $\Delta GMF = -0.015$ respectively), bet hedging continues to exhibit sign inversion. When $\Delta GMF = 0.01$, we expect bet hedging to be unconditionally beneficial, but it is actually deleterious at sufficiently small population sizes. Conversely, when $\Delta GMF = -0.015$ and bet hedging is expected to be unconditionally deleterious, bet hedging is beneficial at sufficiently large population sizes.

How stochasticity in phenotype and environment alter the sign of selection

In order to investigate how different sources of stochasticity play a role in determining the sign of selection, we employed alternative models of both phenotypic distribution and environmental change that allow us to turn "off" these sources of stochasticity independently (see Methods). There are thus four possible combinations, which we designate P1E1, P1E0, P0E1 and P0E0, corresponding respectively to both sources present, phenotypic stochasticity alone, environmental stochasticity alone, and both sources absent. When phenotypic stochasticity is turned "off" (P0), every generation exactly P_{Spec} individuals adopt the specialist phenotype, even if that would require a fraction of an individual. When environmental stochasticity is turned "off" (E0), every generation the environment switches, such that P_A is realized exactly in as few generations as possible. Figure 2 utilized the P1E1 model, as both stochasticity in environment and phenotype are turned "on." In all four models, stochasticity in reproduction is maintained.

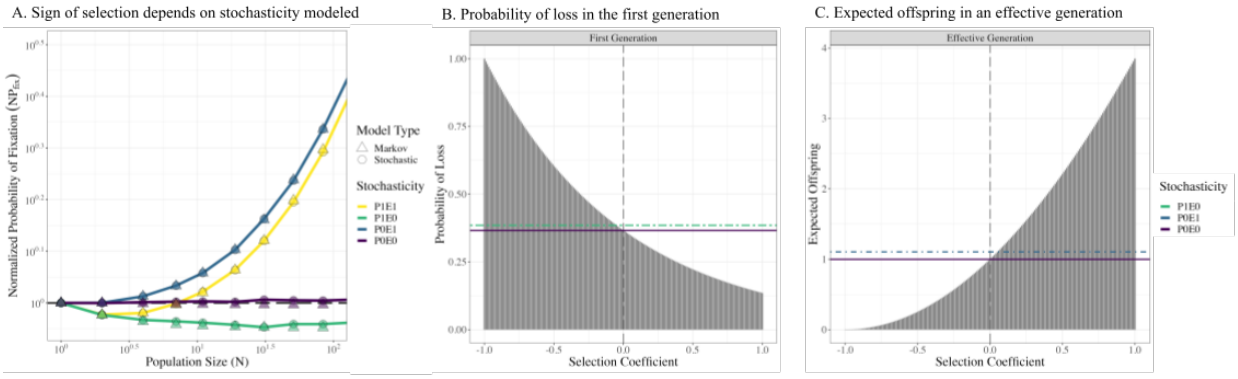

Figure 3: A. We calculate NP_{Fix} for a single invading diversified bet hedging mutant with a $\Delta GMF = 0$, under four models of stochasticity: with and without phenotypic (P1 and P0) and environmental (E1 and E0) stochasticity. In all models, stochasticity in reproduction is maintained. Bet hedging exhibits sign inversion under the P1E1 model, is unconditionally deleterious under P1E0, unconditionally beneficial under P0E1, and selectively neutral under P0E0. B. Probability of loss after one generation in a constant environment as a function of selection coefficient for $N = 100$. Because the relationship between probability of loss and selection coefficient is concave up, increasing within-generation fitness variance increases probability of loss, as seen by the realized probability of loss for the P1E0 model and P0E0 model (green and purple respectively in Panel A). C. The expected number of bet hedger offspring at the end of an effective generation (here two consecutive generations) is also concave up. Therefore, increasing variance in fitness across an effective generation increases the expected offspring as seen by the realized expected offspring for the P0E1 model and P0E0 model (blue and purple respectively in Panel A).

To investigate the mechanisms that cause the sign of selection for the bet hedger to differ from the prediction, we consider $\Delta GMF = 0$, where bet hedging is predicted to be neutral. When both stochasticity in phenotype and environment are modeled (P1E1), bet hedging exhibits sign inversion, as was previously shown in Figure 2 (fig. 3A). Interestingly, when only phenotypic stochasticity is modeled (P1E0), bet hedging is deleterious across all population sizes. The opposite is true when only stochasticity in the environment is modeled (P0E1), in which case bet hedging is beneficial across all population sizes. When neither environmental nor phenotypic stochasticity is considered (P0E0), bet hedging is neutral across all population sizes, which aligns with the deterministic prediction (fig. 3A). This leads us to conclude that stochasticity in phenotype and environment are driving the sign of selection to differ from the prediction based on GMF.

Why does GMF not adequately predict the sign of selection for the bet hedger? GMF is computed using the expectation for both phenotypic luck (i.e. the proportion of offspring which adopt each phenotype) and environmental luck (i.e. proportion of generations spent in each environment). Under the P0E0 model, these expectations are met even at finite population sizes and timescales, hence the sign of selection aligns with the deterministic prediction (fig. 3A, purple). However when both phenotype and environment vary stochastically, the realized luck in phenotypic distribution and environmental regime can differ from the expectation. Bet hedgers that experience better luck than the expectation, whether in phenotypic distribution or environmental regime, will be more likely to reach fixation than the deterministic prediction suggests. Conversely, realizations which experience worse luck will be less likely to reach fixation. If enough realizations experience significantly different luck than the expectation, either better or worse, this can alter the overall probability of fixation for the bet hedger due to nonlinear properties of reproduction.

We utilize the Markov matrices to investigate how realized "luck" in both phenotype and environment alter the probability of fixation for the bet hedger in a single time step when $\Delta GMF = 0$. The Markov matrix model allows us to estimate the probability of fixation by the considering the transition probability for a bet hedger from one frequency to another in a generation. In order to understand these results more deeply, we focus on two metrics calculated from these Markov matrices: probability of loss and expected offspring count (see Methods).

Phenotypic stochasticity alone causes bet hedging to be less beneficial than expected when $\Delta GMF = 0$ by increasing variance in fitness in a single generation (fig. 3A, green). We analyze how phenotypic stochasticity decreases probability of fixation by comparing the P1E0 and P0E0 models, incorporating no environmental stochasticity. The effect of phenotypic stochasticity can be best understood by considering the first generation when there is only a single bet hedging mutant. When phenotypic stochasticity is also turned off (P0E0), the realized phenotypic distribution will always be equal to the expectation. Given that $P_A = 0.5$ and $P_{Spec} = 0.5$, there are two possible scenarios with equal probability: a bet hedger individual with exactly $P_{Spec} = 0.5$ in environment A and be weakly deleterious, or a bet hedger individual with exactly $P_{Spec} = 0.5$ in environment B and be weakly beneficial. On the other hand, stochasticity in phenotypic distribution means that the initial bet hedger may exhibit either the specialist or the conservative phenotype, but not both. As such, there are four possible outcomes under the P1E0 model, each with equal probability: the bet hedger will adopt the specialist phenotype in environment A and be neutral, the bet hedger will adopt the specialist phenotype in environment B and be neutral, the bet hedger will adopt the conservative phenotype in environment A and be strongly deleterious, or the bet hedger will adopt the diversified phenotype in environment B and be strongly beneficial. This means that the variance in realized fitness in the first generation will be greater for a bet hedger under the P1E0 model than for a bet hedger under the P0E0 model.

Increasing variance in fitness in a single generation via phenotypic stochasticity causes bet hedging to be less beneficial than expected by increasing probability of loss. In an invariable environment, the relationship between probability of loss and fitness in a single generation is concave up (fig. 3B). Due to the fact that this function is nonlinear, or more specifically concave up, we can utilize Jensen's Inequality. Jensen's Inequality states that for nonlinear functions, the mean of a function over a set of values differs from the function of the mean of that same set of values. More specifically in our example, the mean probability of loss across a range of fitness values will be greater than the probability of loss for the mean fitness of our range. In other words, the realized probability of loss for the P1 model will be greater than the expectation, thereby causing bet hedging to be less beneficial than expected. Additionally, increasing variance in phenotypic distribution will cause the mean probability of loss to also increase. Indeed, we find that the realized probability of loss for the P1E0 model is greater than that of the P0E0 model

in a single generation (fig. 3B). Additionally, we find no difference in the expected offspring counts in a single generation between the P1 and P0 models (see fig. S1A), indicating that increasing probability of loss is the mechanism that causes the P1 model to be less beneficial than the deterministic prediction.

On the other hand, environmental stochasticity alone causes bet hedging to be more beneficial than expected by increasing variance in fitness across generations (fig. 3A, blue). We analyze how environmental stochasticity increases probability of fixation by comparing the P0E1 and P0E0 models, incorporating no phenotypic stochasticity. Unlike phenotypic stochasticity, environmental stochasticity will not alter realized luck in the first generation, as the environment in the first generation is randomly drawn from A or B for both the P0E0 and P0E1 models. Moreover, environmental variance cannot be measured in any one generation, as only one environmental state will be experienced in any single generation. Therefore, we must look across multiple generations in order for multiple environments, and subsequently GMF, to be realized. In order to do so, we now define an effective generation, or the minimum number of generations in which all environments will be realized in their predicted proportions under the E0 model. As $P_A = 0.5$, here an effective generation is two consecutive generations. For the P0E0 model there are only two possible scenarios over the course of an effective generation, each with equal probability: the regime will be AB and the bet hedger's realized mean fitness will be equal to its GMF, or the environmental regime will be BA and the bet hedger's realized mean fitness will again be equal to its GMF. By contrast, in the P0E1 model, the environment in the second generation is independent of that in the first generation, with either state occurring with equal probability as $P_A = 0.5$. Thus, there are four equally likely scenarios: the regime will be AB or BA and the bet hedger's realized mean fitness will be equal to its GMF, the environmental regime will be AA and the bet hedger will be strongly deleterious, or the regime will BB and the bet hedger will be strongly beneficial.

Increasing variance in fitness across an effective generation via environmental stochasticity causes bet hedging to be more beneficial than expected by increasing expected offspring. This is again due to Jensen's Inequality, as the relationship between fitness and expected offspring after two generations is also concave up (fig. 3C). This again means that the mean expected offspring across a range of different environmental regimes, and thereby fitnesses, will be greater than

the expected offspring of the mean fitness. Indeed, we find that the realized expected number of offspring for the P0E1 model is greater than that of the P0E0 model (fig. 3C). While we also find that the realized probability of loss in an effective generation is less for the P0E1 model than for the P0E0 model, we find that this is due to the relationship between probability of loss and expected offspring, and is therefore not an additional mechanism (see fig. S1B). We therefore conclude that the relationship between fitness variance and expected offspring is the mechanism that causes environmental stochasticity to increase the probability of fixation for the bet hedger.

We now investigate the way phenotypic and environmental stochasticity interact using the P1E1 model, where bet hedging exhibits sign inversion (fig. 3A, yellow). We therefore must consider not only how the two sources of stochasticity interact with each other, but also how they interact with population size. Qualitatively, we can see that the sign of selection for the P1E1 model aligns with the P1E0 model at small population sizes, and the P0E1 model at large population sizes. This is because the relative importance of each source of stochasticity changes differentially with population size.

First, phenotypic stochasticity causes the largest decrease in probability of fixation at small population sizes, and decreases in importance as population size increases (fig.3A, blue line). This is because the phenotypic distribution is pulled from a binomial distribution whose sample size is equal to the number of bet hedger individuals, which means that variance in realized phenotypic distribution will decrease as the number of bet hedger individuals increases. In realizations in which the diversified bet hedger succeeds, the number of bet hedgers will grow large over time, driving down the variance. At small population sizes, the maximum number of bet hedger individuals will remain very small, and for a lineage's entire trajectory to fixation the bet hedger will experience high variance in realized phenotype. We observe that as population size increases, the difference between the probability of fixation for the P1E0 and P0E0s decreases as population size increases (fig.3A, blue line).

Environmental stochasticity causes the largest increase in probability of fixation at large population sizes, and decreases in importance as population size decreases. This is because of the difference in the relative rates of change in the probability of experiencing good environmental luck and the neutral benchmark with population size. Variance in environmental luck, and therefore the probability of experiencing out-sized good environmental luck, is proportional to

the time to fixation / loss. Because time to fixation scales with approximately the natural log of population size, this means that time to fixation will slowly increase as population size increases. As such, even at very large population sizes, time to fixation will be relatively short, and variance in environmental luck will be high enough that favorable environmental regimes are probable. In contrast, the neutral benchmark decreases as population size increases much more quickly (neutral $P_{fix} = 1/N$). Therefore, at small population sizes, when $\Delta GMF = 0$, the probability of experiencing sufficiently good environmental luck is only marginally greater than the neutral benchmark. Therefore, any increase in probability of fixation due to environmental luck is not sufficient to offset the decrease in probability of fixation due to phenotypic luck at small population sizes. But at larger population sizes, where the neutral benchmark is much smaller, the probability of experiencing sufficiently good environmental luck can be much greater than the neutral benchmark, and the bet hedger will be beneficial.

Extending results across parameter space

We extend our previous results across parameter space by varying both P_{Spec} and ΔGMF for all four models of stochasticity: P1E1 (fig. 4A), P0E1 (fig. 4B), P1E0 (fig. 4C) and P0E0 (fig. 4D). The value of P_{Spec} is varied on the horizontal axis, which allows us to relax the previous assumption that $P_{Spec} = 0.5$, thereby investigating the sign of selection for both diversified and conservative bet hedging strategies. Recall that for $P_{Spec} = 0$ the bet hedger exhibits a conservative strategy, for $P_{Spec} = 1$ the bet hedger acts as a wild-type lineage and is thus neutral, and for intermediate values of P_{Spec} the bet hedger exhibits a diversified strategy. ΔGMF is varied on the vertical axis, with $\Delta GMF = 0$, where bet hedging is expected to be neutral, denoted with a blue line. In this way, we are able to cover and extend beyond the range of values of ΔGMF shown in Figure 2. Values of ΔGMF that are impossible to experience in combination with certain values of P_{Spec} are purposefully left blank (see Methods). For each point in parameter space, the sign of selection was characterized as being unconditionally beneficial, exhibiting sign inversion, unconditionally deleterious, or neutral based on the results of stochastic simulations across a range of population sizes from 10^0 to 10^6. For points in parameter space where bet hedging exhibits sign inversion, we interpolate the population size where the NP_{fix} curve crosses the neutral benchmark before becoming beneficial (N_{Crit}). For example, in Figure 2, we observed that the NP_{fix} curve for

$\Delta GMF = 0$ crosses the neutral benchmark at an $N_{Crit} \approx 10$. As our results for both individual-based simulations and Markov Chain numerics align, we show only the results of the individual-based simulations here, since their run times scale more favorably as population size increases.

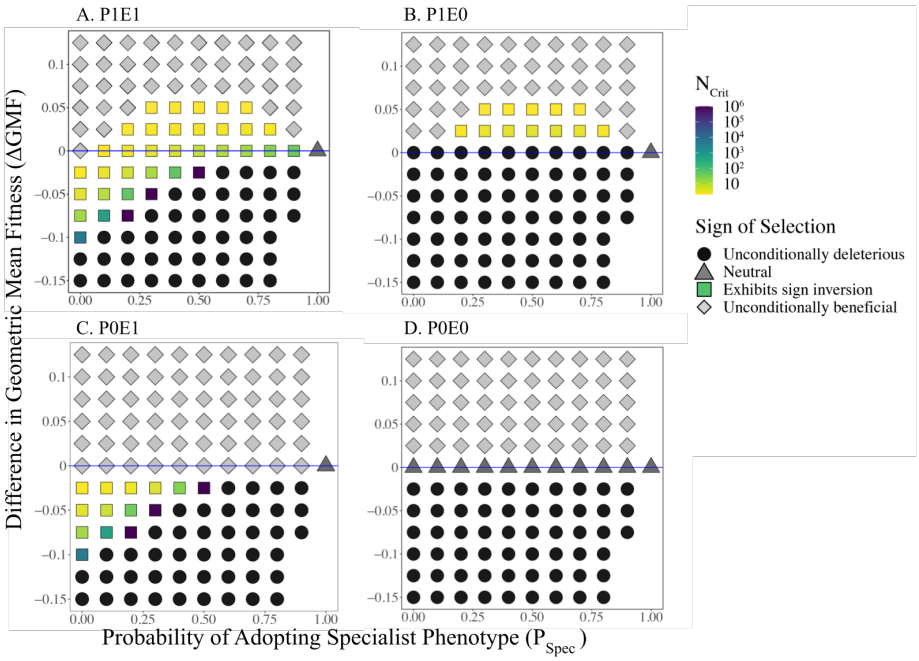

Figure 4: The sign of selection of the bet hedger across parameter space is characterized using stochastic, individual based simulations. The x-axis is P_{Spec}, or the proportion of bet hedger offspring which adopt the specialist phenotype. $P_{Spec} = 0$ corresponds to a conservative bet hedging strategy, $0 < P_{Spec} < 1$ corresponds to a diversified bet hedging strategy, and $P_{Spec} = 1$ corresponds to the specialist wild-type. The y-axis is the difference in geometric mean fitness (ΔGMF) of the bet hedger and the wild-type. Values of ΔGMF that are impossible to achieve in combination with their associated P_{Spec} are intentionally left blank (see Methods). For points in parameter space where bet hedging exhibits sign inversion, N_{Crit} is interpolated. A. Stochasticity in both environment and phenotype are present (P1E1). B. Phenotypic stochasticity alone (P1E0). C. Environmental stochasticity alone (P0E1) D. No stochasticity in either environment or phenotype (P0E0).

When both sources of stochasticity are considered (as was the case in Figure 2), we find that the sign of selection on the diversified bet hedger can differ from the deterministic prediction across parameter space (fig. 4A). In agreement with Figure 3A, bet hedging exhibits sign inversion when $\Delta GMF = 0$. Additionally, the diversified bet hedger exhibits sign inversion when it is predicted to be beneficial ($\Delta GMF > 0$) and deleterious ($\Delta GMF < 0$). In contrast, because the conservative bet hedger does not experience phenotypic stochasticity, we only see sign inversion

when the bet hedger is expected to be deleterious ($\Delta GMF < 0$).

When bet hedging exhibits sign inversion, we find that the value of N_{Crit} increases as the ΔGMF decreases. In other words, the range of population sizes where bet hedging is deleterious increases as ΔGMF decreases. This is unsurprising, as decreasing GMF is expected to cause the probability of fixation to decrease across all population size. This shifting the NP_{fix} curve downward will in turn increase N_{Crit}.

When only stochasticity in phenotype is turned on (fig. 4B), we find that our results disagree with the deterministic prediction only when $\Delta GMF \geq 0$. Under the P1E0 Model, both conservative and diversified bet hedging are unconditionally deleterious when $\Delta GMF = 0$ (i.e. fig. 3A). Diversified bet hedging also exhibits sign inversion for some values when $\Delta GMF > 0$; the sign of selection differs from the deterministic prediction only at small population sizes. Because conservative bet hedging experiences no phenotypic stochasticity, the conservative bet hedger does not exhibit sign inversion here. This mirrors our previous finding that stochasticity in phenotype causes bet hedging to be less beneficial than expected, and that it has the largest impact on the probability of fixation at small population sizes (fig. 3A). Turning on phenotypic stochasticity causes bet hedging to become deleterious when the parameters are such that the decrease in P_{fix} due to phenotypic stochasticity is greater than the probability of fixation under the P0E0 model. As population size increases when $\Delta GMF > 0$, the cost of phenotypic stochasticity decreases while the probability of fixation under the P0E0 model increases. As we increase ΔGMF, the probability of fixation under the P0E0 model at any population size will increase, thus decreasing the population sizes where bet hedging is deleterious. We find $N_{Crit} \approx [2, 20]$ in this region of parameter space. Due to the relationship between ΔGMF and N_{Crit}, if sign inversion were to extend to greater ΔGMF than the range we found, we would expect $N_{Crit} < 2$, which is numerically impossible. Therefore, because both the minimum and maximum values of N_{Crit} observed are well within the range of population sizes surveyed, we are confident that sign inversion does not occur in additional regions of parameter space.

When only stochasticity in the environment is turned on (fig. 4C), we find that our results disagree with the deterministic prediction only when $\Delta GMF \leq 0$. Under the P0E1 Model, both conservative and diversified bet hedging are unconditionally beneficial when $\Delta GMF = 0$ (i.e. fig. 3A). Both conservative and diversified bet hedging also exhibit sign inversion for some values of

$\Delta GMF < 0$, or changes the sign of selection exclusively at large population sizes. Environmental stochasticity alters the sign of selection when the probability of experiencing sufficiently good environmental luck is greater than the neutral benchmark. As ΔGMF decreases, the amount of good environmental luck needed for the bet hedger to fare better than the neutral expectation will increase, and therefore become less probable. This in turn means that the probability of fixation will only cross the neutral benchmark at larger population sizes, where the neutral benchmark is smaller. We find N_{Crit} varies between 55 and 10^6 in this region of parameter space. If sign inversion were to occur in smaller ΔGMF than the range shown here, we would expect to see $N_{Crit} >> 10^6$, which is beyond the maximum population size surveyed. Therefore, it is possible that sign inversion could occur at some points currently labelled as being unconditionally deleterious. However, because N_{Crit} increases exponentially, extending the maximum population size surveyed to capture this is not feasible (fig. S2).

When both stochasticity in the phenotype and environment are turned off (fig. 4D), the sign of selection for the bet hedger aligns exactly with the deterministic predictions across all parameter space. We find that bet hedging is unconditionally beneficial when $\Delta GMF > 0$, neutral when $\Delta GMF = 0$, and unconditionally deleterious when $\Delta GMF < 0$.

Discussion

Bet hedging is an adaptation to unpredictable, random environmental change. We find that deterministic treatments (namely, predictions based on the GMF) do not always adequately capture the sign of selection for bet hedging. We have developed a stochastic framework that captures the effects of variance in phenotype, environment, and reproduction, thereby gaining additional insight into when and why bet hedging will evolve. Using both stochastic individual-based simulations and Markov numeric matrices, we find that explicitly incorporating stochasticity changes the sign of selection of bet hedging compared to well-established, deterministic results. We find when bet hedging is expected to be beneficial given its GMF, instead bet hedging can be deleterious at sufficiently small population sizes. In contrast, when bet hedging is expected to be deleterious given its GMF, bet hedging can actually be beneficial at sufficiently large population sizes. This is notable for two reasons: first, the deterministic predictions does not adequately capture the sign of selection; and second, the sign of selection can change with population size.

This dependence on population size is known as sign inversion (Raynes et al. 2018), and means that a bet hedger can be deleterious at small population sizes but beneficial at larger population sizes. Our results represent the first observation of sign inversion in a model of bet hedging.

We discover that stochasticity in phenotype and environment cause the sign of selection to differ from the deterministic prediction. When stochasticity in neither phenotypic distribution nor environment are considered, the probability of fixation aligns with the deterministic prediction; bet hedging is unconditionally beneficial when the GMF of the bet hedger is greater than the wild-type, neutral when the GMF of the bet hedger is equal to that of the wild-type, and unconditionally deleterious when the GMF of the bet hedger is less than that of the wild-type. Moreover, the two sources of stochasticity alter the sign of selection in opposing ways due to the time scales upon which they operate. Stochasticity in phenotypic distribution causes bet hedging to be less adaptive than deterministic methods predict by increasing variance in within-generation fitness, thereby increasing the bet hedger's probability of loss. Phenotypic stochasticity acts most strongly at small population sizes, when the number of bet hedger individuals remains small and therefore variance in realized phenotypic distribution is high. By definition, only diversified bet hedgers will suffer the cost of phenotypic stochasticity; conservative bet hedgers experience no variance in realized phenotype. Because of this, we predict that conservative bet hedging will be more adaptive than diversified bet hedging when effective population size is small. On the other hand, stochasticity in the environmental regime causes bet hedging to be more adaptive than the deterministic models predict by increasing variance in between-generation luck, which causes an increase in expected offspring number. Stochasticity in the environment acts most strongly at large population sizes, where the probability of experiencing sufficiently good environmental luck can be much greater than the neutral benchmark.

The evolution of conservative vs. diversified bet hedging strategies is an ongoing question in the field. Our results suggest that conservative bet hedging may be more beneficial in a larger range of contexts, yet observations of diversified bet hedging in nature far exceed observations of conservative bet hedging (Simons 2011). This could be due to observational bias; diversified bet hedging is more likely to be studied as high phenotypic variance is easier to notice, and different definitions of bet hedging sometimes exclude conservative bet hedging (de Jong et al. 2011, Grimbergen et al. 2015, Morawska et al. 2022). It is also true that not all examples of bet hedging

align with our framework of diversified and conservative bet hedging existing on a continuum of P_{Spec}. In this framing, some proportion of offspring adopt a specialist phenotype that has a high fitness in the first environment, while the remainder adopt the generalist phenotype (Childs et al. 2010, Altiero et al. 2010, Byrne and Keogh 2009). Venable 1985 posited that diversified bet hedging strategies could be subdivided into two categories based on the second phenotype: those in which the second phenotype is "low risk" (i.e. the conservative phenotype), and those in which the second phenotype is "high risk" (i.e. a specialist adapted to the second environment). For diversified bet hedgers of the second class, where both phenotypes are high risk, there exists no corresponding conservative strategy. For example, in the delayed seed germination strategy, having all seeds germinate in the second year is no more conservative than having all seeds germinate in the first year (Venable 1985, Venable 2007).

Other possible explanations for the lack of observed examples of conservative bet hedging relate to the evolutionary accessibility of each bet hedging strategy. It is possible that there are fitness side-effects of the conservative phenotype not captured by our model; for example, the conservative phenotype in plant mating systems is cleistogamous, or asexual, flowers, which carry the risks associated with inbreeding (Culley and Klooster 2007). Additionally, we do not explicitly consider the phylogenetic history of bet hedging. In our model, we assume the specialist, non bet hedging strategy is the ancestral wild-type. This appears to be the case in some examples: cleistogamy in annual plants (Culley and Klooster 2007), bacterial persistence (Beaumont et al. 2009), and seed heteromorphism (Scholl et al. 2020). However there have been a lack of phylogenetic analyses of bet hedging evolution to confirm that the specialist is universally the ancestral state. Yet another possible explanation for the dominance of diversified bet hedging in nature could be that on a molecular level, suppressing the cellular noise that leads to phenotypic variance (i.e. diversified bet hedging) is virtually impossible (Richard and Yvert 2014). This would mean that for both conservative bet hedging and specialist strategies, having no variance in realized phenotype (or a P_{Spec} of precisely 0 or 1 respectively) would never evolve. This is certainly the case for bacterial persistence, as both "specialist" and "conservative" lineages exhibit very low rates of phenotypic switching (Van den Bergh et al. 2016). In the future, we plan to expand our model to better predict the drivers of bet hedging strategy evolution.

Our finding that environmental stochasticity causes deterministic predictions to break down

has broad implications for evolution in changing environments. Bet hedging is often discussed in comparison to two other strategies for coping with changing environments: adaptive tracking and phenotypic plasticity (Simons 2011). Adaptive tracking is evolution by selection on standing genetic variation, and is predicted to be only adaptive when the time-scale of environmental change is very slow. In other words, adaptive tracking is analogous to evolution by natural selection in a constant environment (Graves and Weinreich 2017, Simons 2011). Therefore, we predict that the evolution of adaptive tracking, unlike bet hedging evolution, is likely to follow deterministic predictions. Phenotypic plasticity, on the other hand, is an adaptation to rapid environmental change where an organism can modulate its phenotype in response to some environmental cue (Schlichting and Pigliucci 1998). Phenotypic plasticity is predicted to evolve when environmental change is rapid and provides predictable cues; the organism must be able to change in response to environmental stimuli Simons 2011. Similar to bet hedging, phenotypic plasticity is expected to evolve in fast changing environments. Additionally, while phenotypic plasticity is thought of as distinct from diversified bet hedging, the two strategies are often linked. The reaction norms that cause an individual to adopt a certain phenotype in response to an environmental cue can be noisy such that there is phenotypic heterogeneity within a single generation (Simons 2011). Therefore, phenotypic plasticity will experience all three sources of stochasticity that shape bet hedging evolution: in reproduction, in phenotype, and in environment. Thus, it seems likely that the sign of selection for phenotypic plasticity will similarly differ from the deterministic prediction. Future work that investigates the evolution of plasticity, the relationship between diversified bet hedging and plasticity, and the effect of environmental predictability could illuminate additional ways in which deterministic predictions fail in changing environments.

Our results also fit into a larger conversation about the evolution of lineage-variable traits, or alleles whose fitness can change between carriers (Graves and Weinreich 2017, Raynes et al. 2021). In contrast to directly selected mutations, i.e. alleles with a constant fitness across all contexts, lineage-variable traits will experience variance in fitness either within a single lineage, such as alleles that increase cooperation (Nowak et al. 2004) and recombination (Whitlock et al. 2016, or between lineages, such as alleles that increase mutation rate (Raynes et al. 2018). All other traits that exhibit sign inversion have been found to have lineage-variable fitness effects,

yet other commonalities between lineage-variable traits are still being explored (Raynes et al. 2021). Our results provide an additional example of lineage-variable selection to the growing canon. Also, bet hedging is unique among other lineage-variable traits because it displays both within-lineage fitness variance (i.e. variance in realized phenotype) and between-lineage fitness variance (i.e. variance in realized environmental regime) simultaneously. Our work shows that these two types of lineage-variable selection may not be mutually exclusive, and provides some preliminary insights into how within- and between- lineage variance can interact.

Our generalized model is able to predict the adaptive significance of bet hedging. Ongoing work of ours to capture other intricacies of real-world bet hedging, such as spatial environmental change (Starrfelt and Kokko 2012, Martín et al. 2019), will allow us to explore further ways stochasticity can change the sign of selection. An existing problem in the field of bet hedging research has been discerning the adaptive significance of putative bet hedging traits; in 2011, Simons found that only 10% of bet hedging studies were able to demonstrate that the putative bet hedging trait was adaptive in its current environmental regime (Simons 2011). Determining whether a bet hedging trait is adaptive in its current environmental context can be challenging, both from an experimental and theoretical perspective. Here, we prove that GMF alone does not capture the full extent to which bet hedging is adaptive. We introduce a new framework that could be applied to a range of real-world examples of bet hedging to prove their adaptive significance.

Methods

Stochastic individual based simulations

We model the evolution of bet hedging in asexual populations of constant size N evolving in discrete non-overlapping generations under the Wright-Fisher model. Simulations start with N individuals, consisting of a single invading bet hedging mutant and $N-1$ resident wild-type individuals, and end when the bet hedger reaches fixation (frequency of 100%) or goes extinct (frequency of 0%). Every generation, the environment adopts one of two states: $Environment A$, or the stress-free condition, with probability P_A and $Environment B$, or the stress condition, with probability $1-P_A$. Henceforth, we will keep $P_A = 0.5$ constant, as our results are not sensitive to

the value of P_A. The wild-type phenotype has a higher fitness in A ($w_A = 2$), and a much lower fitness in B ($w_B = 0.5$). The GMF of the wild-type is thus $2^{0.5} \times 0.5^{0.5} = 1$. We describe the fitness of the bet hedger next.

We introduce a framework to characterize conservative bet hedging, diversified bet hedging, and the wild-type specialist strategy as a continuum of a single parameter: the probability offspring will adopt the specialist phenotype, or P_{Spec}. When $P_{Spec} = 0$, all offspring will adopt the conservative phenotype, and the lineage will exhibit a conservative bet hedging strategy. When $P_{Spec} = 1$, all offspring will adopt the specialist phenotype, and therefore the lineage will exhibit the wild-type specialist strategy. For intermediate values of P_{Spec}, or mixed strategies, the lineage will exhibit a diversified bet hedging strategy (fig1B).

The bet hedging population is defined by two parameters: P_{Spec}, the proportion of offspring that adopt the specialist phenotype, and w_C, the fitness of the conservative phenotype in both environments. From these two parameters, we can calculate the GMF of the bet hedger as:

$$\left(P_{Spec} * w_A + (1 - P_{Spec}) * w_C\right)^{P_A} * \left(P_{Spec} * w_B + (1 - P_{Spec}) * w_C\right)^{1-P_A}$$

For a conservative bet hedger, since $P_{Spec} = 0$, the GMF will be simply w_C. Parameters are selected such that the bet hedger always has both a lower AMF and temporal fitness variance than the resident wild-type. We standardize the GMF of the bet hedger by the GMF of the wild-type. We characterize ΔGMF as the difference in fitness between the fitness of the bet hedger and the wild-type divided by the fitness of the wild-type, similar to a selection coefficient in classic population genetics models. Therefore, under deterministic assumptions, when $\Delta GMF > 1$ bet hedging is expected to be beneficial, when $\Delta GMF = 0$ bet hedging is expected to be neutral, and when $\Delta GMF < 1$ bet hedging is expected to be deleterious.

Reproduction is simulated according to the Wright–Fisher model, where the number of offspring in the next generation is drawn from a multinomial distribution with the expectation determined by each lineage's current frequency multiplied by the lineage's relative fitness in that generation.

The probability of fixation is calculated as the proportion of replicates in which the bet hedger reaches fixation. The neutral expectation, or the probability of fixing for a neutral allele, is equal to its starting frequency ($1/N$). As such, we follow others (Raynes et al. 2018) and normalize the

probability of fixation by the neutral expectation ($P_{Fix}/(1/N)$), so that probabilities of fixation above neutral (or beneficial) will be greater than one, and probabilities of fixation below neutral (or deleterious) will be less than one. Simulations are repeated across a range of different values of P_{Spec} and w_C. Simulations are carried out across 20 population sizes evenly spaced on a logarithmic scale between $N = 10^0$ and $N = 10^6$.

Additionally, we utilize different models of both phenotypic and environmental stochasticity, in order to independently turn each type of stochasticity "on" and "off". Henceforth, we designate these stochasticity types as being turned on with "1" and off with "0." Therefore, the model where both phenotypic and environmental stochasticity are turned on would be designated P1E1. We detail both the phenotypic and environmental models of stochasticity next.

Individual based simulations are carried out in the Julia Programming Language (Bezanson et al. 2017) using the Distributions Package (Besançon et al. 2021).

Implementing phenotypic stochasticity in individual based simulations

Phenotypic stochasticity can be either turned on (P1) or turned off (P0). Under the P1 model, every generation the realized phenotypic distribution is pulled from a binomial distribution with probability equal to P_{Spec} and sample size equal to the number of bet hedger individuals in the population.

Under the P0 model, every generation the realized phenotypic distribution is exactly equal to the expectation (P_{Spec}).

Implementing environmental stochasticity in individual based simulations

Similarly, we utilize two models of environmental stochasticity: one where environmental stochasticity is turned on (E1) on one where environmental stochasticity is turned off (E0). Under the E1 model, every generation the environmental state is randomly assigned to either Environment A (with probability P_A), or Environment B (with probability $1 - P_A$).

Under the E0 model, the environment switches deterministically every generation. In the first generation, the environment is still randomly assigned to either Environment A (with probability P_A), or Environment B (with probability $1 - P_A$). In subsequent generations, the environment assigned is opposite to the environment in the previous generation.

Markov matrix numeric model

We also use a Markov numeric matrix model to compute the probability of fixation for a single bet hedger in a population of $N-1$ wild-type individuals.

First, single generation transition matrices are constructed for each possible environment (T_A and T_B). Transition matrices depend on two parameters: the relative fitness of the bet hedger and the population size. Transition matrices are $N+1 \times N+1$ matrices that give the probability of transitioning from all possible values of starting frequency, x_t, to all possible values of ending frequency x_{t+1} in a single time step. Each column of the matrix corresponds to a different starting frequency of x_t, while each row corresponds to a different ending frequency of x_{t+1}. The matrix can therefore be written out as:

$$T = \begin{bmatrix} P(x_t = 0|x_{t+1} = 0) & P(x_t = 1/N|x_{t+1} = 0) & \cdots & P(x_t = N|x_{t+1} = 0) \\ P(x_t = 0|x_{t+1} = 1/N) & P(x_t = 1/N|x_{t+1} = 1/N) & \cdots & P(x_t = N|x_{t+1} = 1/N) \\ \cdots & \cdots & \ddots & \cdots \\ P(x_t = 0|x_{t+1} = N) & P(x_t = 1/N|x_{t+1} = N) & \cdots & P(x_t = N|x_{t+1} = N) \end{bmatrix}$$

In order to adequately capture selection across changing environments, we must consider the impact of environmental regime, or the sequence of environmental states experienced. Because GMF is a measure of fitness across environments, and therefore generations, it cannot be realized in any single generation. As such, we introduce an effective generation matrix. We define an effective generation as the minimum number of generations necessary for all environments to be experienced with their expected proportion (P_A). In our case, as $P_A = 0.5$, an effective generation is two generations. For example, the environmental regime experienced during an effective generation could be AB or BA. The effective generation regime matrices are therefore calculated as the product of the transition matrices for the first and second generations ($T_{EnvinGeneration=2} \times T_{EnvinGeneration=1}$). This yields a matrix that will give the expected transition probabilities at the end of the effective generation. Effective generation regime matrices are computed separately for each possible environmental regime (e.g. AB, BA, etc.). Next, we calculate the effective generation matrix as the arithmetic mean of all possible environmental regime matrices. As each regime is equally likely to occur and individual regimes are being experienced by separate

realizations, the arithmetic mean is used.

Finally, the probability of fixation is calculated by estimating the stationary distribution of the effective generation matrix. The stationary distribution (π) is the matrix that satisfies the condition $\pi = \pi \times T$. Probability of fixation is the $P(x_t = 1/N | x_{t+1} = N)$ entry of the stationary distribution matrix.

We also calculate two summary statistics from our single generation and effective generation matrices in order to understand the dynamics of these models in a single time step: probability of loss and expected number of bet hedger offspring. Probability of loss is simply the probability of the bet hedger having a frequency of 0 at the end of the time step, or the $P(x_t = 1/N | x_{t+1} = 0)$ entry of the matrix. Expected offspring is calculated as the mean number of offspring a single bet hedger will have at the end of the time step, or the sum of the $x_t = 1/N$ column.

As was the case with the stochastic individual-based simulations, we utilize different models of both phenotypic and environmental stochasticity, in order to independently turn each type of stochasticity "on" (1) and "off" (0). We detail the implementation of the phenotypic and environmental models of stochasticity in the Markov matrix model next.

Matrix multiplication was carried out using the MATLAB Programming Language (MATLAB 2010).

Implementing phenotypic stochasticity in the Markov matrix model

Phenotypic stochasticity can change the realized fitness of the bet hedger in any single generation. Under the P1 model, the realized phenotypic distribution of the bet hedger can differ from the expectation, with variance modulated by the number of bet hedger individuals present in the population. As variance is modulated by the number of bet hedgers present, or x_t, we must calculate all possible values of realized P_{Spec} for every column in our matrix individually using a binomial distribution with probability equal to P_{Spec} and sample size equal to x_t. For each possible value of realized P_{Spec}, we then calculate 1) the probability of realizing said P_{Spec}, and 2) all transition probabilities of reaching a frequency of x_{t+1} in the next time step, given the fitness of the bet hedger associated with said P_{Spec}. We then take the weighted average of all possible transition probabilities for the x_t column. We repeat this for all columns, until we have a full transition matrix for both environmental conditions.

Under the P0 model, the realized phenotypic distribution of the bet hedger is exactly equal to the expectation (P_Spec). Thus, the fitness of the bet hedger is assumed to be constant, and independent of bet hedger count, for each of the environments.

Implementing environmental stochasticity in the Markov matrix model

Environmental stochasticity changes the exact sequence of environments experienced by the bet hedger, and therefore the environmental regimes possible within a single effective generation. Under the E1 model, there are four possible environmental regimes for the effective generation: $AB, BA, AA, orBB$. The effective generation matrix is therefore the arithmetic mean of the $T_B \times T_A$, $T_A \times T_B$, $T_A \times T_A$, and $T_B \times T_B$ regime matrices.

Under the E0 model, as the environment switches every generation, there are only two possible environmental regimes for the effective generation: $ABorBA$. It follows that the effective generation matrix will be the arithmetic mean of the $T_B \times T_A$ and $T_A \times T_B$ regime matrices.

Constructing parameter space surveys

We utilize our previous stochastic, individual-based simulations to construct a large scale parameter space survey for all four models of stochasticity (P1E1, P0E1, P1E0, and P0E0). As our previous results for both individual-based simulations and Markov Chain numerics align, we show only the results of the individual-based simulations here, since their run times scale more favorably as population size increases. For every point in parameter space, simulations were carried out at 20 different populations sizes spaced evenly on a logarithmic scale between 10^0 and 10^6 to construct a normalized probability of fixation curve.

We vary the value of $P_{Spec} = [0, 1]$ to relax the previous assumption that $P_{Spec} = 0.5$ and investigate the sign of selection for both diversified and conservative bet hedging strategies. We also vary the value of $\Delta GMF = [-0.15, 0.125]$, so that we can cover values of ΔGMF where bet hedging is expected to be deleterious, neutral, and beneficial. In order to vary ΔGMF, we calculate the corresponding value of w_C that will yield the appropriate ΔGMF given the value of P_{Spec}. However, for certain values of P_{Spec}, there is no possible value of w_C that will yield some values of ΔGMF. For example, when $P_{Spec} = 1.0$, no individuals will adopt the conservative phenotype, and therefore it is impossible to have a value of $\Delta GMF \neq 0$. For combinations of

parameter values that are impossible to experience, the point in parameter space is intentionally left blank.

Characterizing the sign of selection of Probability of Fixation curves

After calculating the normalized probability of fixation (NP_{Fix}) as a function of population size to a maximum population size of 10^6, the vector of all NP_{Fix} values is classified based on whether the sign of selection is unconditionally beneficial, unconditionally deleterious, neutral, or exhibits sign inversion computationally as follows. First, at each population size, the probability of fixation is evaluated as being significantly different from the neutral expectation using a $p < 0.01$ confidence interval. The confidence interval is found by constructing a binomial probability distribution with probability equal to the probability of fixation for a neutral mutation and a number of trials equal to the number of replicates. Then, each NP_{Fix} vector is evaluated based on two criteria: the location of points and the slope. A vector is labeled neutral if all points are not significantly different from the neutral expectation, or 1. A curve is classified as beneficial if all points on the curve are greater than or equal to 1. Similarly, a curve is labeled deleterious if all points are less than or equal to 1. A curve is labeled as exhibiting sign inversion if a) after $N = 1$, the sign of selection changes only once (i.e. from deleterious to beneficial, or from beneficial to deleterious), b) if the sign of the slope of the curve changes only once. Curves that exhibit sign inversion are labelled as concave up if $NP_{Fix}[N = 10^6] > 1$, and concave down if $NP_{Fix}[N = 10^6] < 1$. As we do not find any points in parameter space where the sign of selection is "sign inversion (concave down)", we henceforth shorten the label for points that exhibit "sign inversion (concave up)" as just "sign inversion." NP_{Fix} vectors that do not meet any of these criteria are labeled as ambiguous by the computer and classified manually.

Interpolating the value of N_{Crit}

For curves that exhibit sign inversion, we interpolate the value of N_{Crit}, or the critical population size where the probability of fixation crosses the neutral expectation, using the Linear Interpolation function from the Interpolations Library in the Julia Programming Language (Bezanson et al. 2017.

Statistical analyses

Statistical analyses were carried out using the R Programming Language (Yumpu.com 2010).

Code availability

Simulation code is available at `https://github.com/mweissman97/bethedging_stochastic`.

Funding

MRW was a trainee supported under the Brown University Predoctoral Training Program in Biological Data Science (NIH T32 GM128596). This research was also supported by an EEOB Doctoral Dissertation Enhancement Grant from the Bushnell Graduate Research and Education Fund.

Literature Cited

T. Altiero, R. Bertolani, and L. Rebecchi. Hatching phenology and resting eggs in tardigrades. *Journal of Zoology*, 280(3):290–296, 2010. ISSN 1469-7998. doi: 10.1111/j.1469-7998.2009.00664.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-7998.2009.00664.x. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-7998.2009.00664.x.

L. E. Bagamery, Q. A. Justman, E. C. Garner, and A. W. Murray. A Putative Bet-Hedging Strategy Buffers Budding Yeast against Environmental Instability. *Current Biology*, 30(23):4563–4578.e4, Dec. 2020. ISSN 0960-9822. doi: 10.1016/j.cub.2020.08.092. URL https://www.sciencedirect.com/science/article/pii/S0960982220312823.

N. Q. Balaban, J. Merrin, R. Chait, L. Kowalik, and S. Leibler. Bacterial Persistence as a Phenotypic Switch. *Science*, 305(5690):1622–1625, Sept. 2004. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1099390. URL https://science.sciencemag.org/content/305/5690/1622. Publisher: American Association for the Advancement of Science Section: Report.

N. Q. Balaban, S. Helaine, K. Lewis, M. Ackermann, B. Aldridge, D. I. Andersson, M. P. Brynildsen, D. Bumann, A. Camilli, J. J. Collins, C. Dehio, S. Fortune, J.-M. Ghigo, W.-D. Hardt, A. Harms, M. Heinemann, D. T. Hung, U. Jenal, B. R. Levin, J. Michiels, G. Storz, M.-W. Tan, T. Tenson, L. Van Melderen, and A. Zinkernagel. Definitions and guidelines for research on antibiotic persistence. *Nature Reviews Microbiology*, 17(7):441–448, July 2019. ISSN 1740-1534. doi: 10.1038/s41579-019-0196-3. URL https://www.nature.com/articles/s41579-019-0196-3. Number: 7 Publisher: Nature Publishing Group.

H. J. E. Beaumont, J. Gallie, C. Kost, G. C. Ferguson, and P. B. Rainey. Experimental evolution of bet hedging. *Nature*, 462(7269):90–93, Nov. 2009. ISSN 1476-4687. doi: 10.1038/nature08504. URL https://www.nature.com/articles/nature08504. Number: 7269 Publisher: Nature Publishing Group.

M. Besançon, T. Papamarkou, D. Anthoff, A. Arslan, S. Byrne, D. Lin, and J. Pearson. Distributions.jl: Definition and Modeling of Probability Distributions in the JuliaStats Ecosystem. *Journal of Statistical Software*, 98:1–30, July 2021. ISSN 1548-7660. doi: 10.18637/jss.v098.i16. URL https://doi.org/10.18637/jss.v098.i16.

J. Bezanson, A. Edelman, S. Karpinski, and V. B. Shah. Julia: A Fresh Approach to Numerical Computing. *SIAM Review*, 59(1):65–98, Jan. 2017. ISSN 0036-1445. doi: 10.1137/141000671. URL https://epubs.siam.org/doi/10.1137/141000671. Publisher: Society for Industrial and Applied Mathematics.

C. A. Botero, F. J. Weissing, J. Wright, and D. R. Rubenstein. Evolutionary tipping points in the capacity to adapt to environmental change. *Proceedings of the National Academy of Sciences*, 112(1):184–189, Jan. 2015. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1408589111. URL https://www.pnas.org/content/112/1/184. Publisher: National Academy of Sciences Section: Biological Sciences.

P. G. Byrne and J. S. Keogh. Extreme sequential polyandry insures against nest failure in a frog. *Proceedings of the Royal Society B: Biological Sciences*, 276(1654):115–120, Jan. 2009. ISSN 0962-8452. doi: 10.1098/rspb.2008.0794. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2614246/.

P. Capilla-Lasheras, X. Harrison, E. M. Wood, A. J. Wilson, and A. J. Young. Altruistic bet-hedging and the evolution of cooperation in a Kalahari bird. *Science Advances*, 7(39):eabe8980, Sept. 2021. doi: 10.1126/sciadv.abe8980. URL https://www.science.org/doi/full/10.1126/sciadv.abe8980. Publisher: American Association for the Advancement of Science.

D. Z. Childs, C. J. E. Metcalf, and M. Rees. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society B: Biological Sciences*, 277(1697):3055–3064, Oct. 2010. doi: 10.1098/rspb.2010.0707. URL https://royalsocietypublishing.org/doi/10.1098/rspb.2010.0707. Publisher: Royal Society.

T. M. Culley and M. R. Klooster. The Cleistogamous Breeding System: A Review of Its Frequency, Evolution, and Ecology in Angiosperms. *Botanical Review*, 73(1):1–30, 2007. ISSN 0006-8101. URL https://www.jstor.org/stable/27571184. Publisher: New York Botanical Garden Press.

I. G. de Jong, P. Haccou, and O. P. Kuipers. Bet hedging or not? A guide to proper classification of microbial survival strategies. *BioEssays*, 33(3):215–223, 2011. ISSN 1521-1878. doi:

10.1002/bies.201000127. URL https://onlinelibrary.wiley.com/doi/abs/10.1002/bies.201000127. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/bies.201000127.

S. Einum and I. A. Fleming. Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evolutionary Ecology Research*, 6(3):443–455, 2004. ISSN 1522-0613. URL http://www.evolutionary-ecology.com/abstracts/v06/1657.html. Publisher: Evolutionary Ecology, Ltd.

S. Frank and M. Slatkin. Evolution in a Variable Environment. *American Naturalist - AMER NATURALIST*, 136, Aug. 1990. doi: 10.1086/285094.

M. Gianella, K. J. Bradford, and F. Guzzon. Ecological, (epi)genetic and physiological aspects of bet-hedging in angiosperms. *Plant Reproduction*, 34(1):21–36, Mar. 2021. ISSN 2194-7961. doi: 10.1007/s00497-020-00402-z. URL https://doi.org/10.1007/s00497-020-00402-z.

J. H. Gillespie. NATURAL SELECTION FOR WITHIN-GENERATION VARIANCE IN OFFSPRING NUMBER. *Genetics*, 76(3):601–606, Mar. 1974. ISSN 1943-2631. doi: 10.1093/genetics/76.3.601. URL https://doi.org/10.1093/genetics/76.3.601.

C. J. Graves and D. M. Weinreich. Variability in Fitness Effects Can Preclude Selection of the Fittest. *Annual Review of Ecology, Evolution, and Systematics*, 48(1):399–417, 2017. doi: 10.1146/annurev-ecolsys-110316-022722. URL https://doi.org/10.1146/annurev-ecolsys-110316-022722. _eprint: https://doi.org/10.1146/annurev-ecolsys-110316-022722.

A. J. Grimbergen, J. Siebring, A. Solopova, and O. P. Kuipers. Microbial bet-hedging: the power of being different. *Current Opinion in Microbiology*, 25:67–72, June 2015. ISSN 13695274. doi: 10.1016/j.mib.2015.04.008. URL https://linkinghub.elsevier.com/retrieve/pii/S1369527415000521.

T. R. Haaland, J. Wright, and I. I. Ratikainen. Bet-hedging across generations can affect the evolution of variance-sensitive strategies within generations. *Proceedings of the Royal Society B: Biological Sciences*, 286(1916):20192070, Nov. 2019. doi: 10.1098/rspb.2019.2070. URL https://royalsocietypublishing.org/doi/10.1098/rspb.2019.2070. Publisher: Royal Society.

K. Hopper, J. Rosenheim, T. Prout, and S. Oppenheim. Within-generation bet hedging: a seductive explanation? *Oikos*, 101(1):219–222, 2003. ISSN 1600-0706. doi: 10.1034/j.1600-0706.2003.12051.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1034/j.1600-0706.2003.12051.x. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1034/j.1600-0706.2003.12051.x.

M. Kimura. On the Probability of Fixation of Mutant Genes in a Population. *Genetics*, 47(6): 713–719, June 1962. ISSN 0016-6731. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1210364/.

E. Libby and W. C. Ratcliff. Shortsighted Evolution Constrains the Efficacy of Long-Term Bet Hedging. *The American Naturalist*, 193(3):409–423, Mar. 2019. ISSN 0003-0147, 1537-5323. doi: 10.1086/701786. URL https://www.journals.uchicago.edu/doi/10.1086/701786.

P. V. Martín, M. A. Muñoz, and S. Pigolotti. Bet-hedging strategies in expanding populations. *PLOS Computational Biology*, 15(4):e1006529, Apr. 2019. ISSN 1553-7358. doi: 10.1371/journal.pcbi.1006529. URL https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006529. Publisher: Public Library of Science.

MATLAB. *version 7.10.0 (R2010a)*. The MathWorks Inc., 2010.

D. McAlister and F. Galton. XIII. The law of the geometric mean. *Proceedings of the Royal Society of London*, 29(196-199):367–376, Jan. 1997. doi: 10.1098/rspl.1879.0061. URL https://royalsocietypublishing.org/doi/abs/10.1098/rspl.1879.0061. Publisher: Royal Society.

L. P. Morawska, J. A. Hernandez-Valdes, and O. P. Kuipers. Diversity of bet-hedging strategies in microbial communities—Recent cases and insights. *WIREs Mechanisms of Disease*, 14(2):e1544, 2022. ISSN 2692-9368. doi: 10.1002/wsbm.1544. URL https://onlinelibrary.wiley.com/doi/abs/10.1002/wsbm.1544. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/wsbm.1544.

M. A. Nowak, A. Sasaki, C. Taylor, and D. Fudenberg. Emergence of cooperation and evolutionary stability in finite populations. *Nature*, 428(6983):646–650, Apr. 2004. ISSN 1476-4687. doi: 10.1038/nature02414.

H. Olofsson, J. Ripa, and N. Jonzén. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society B: Biological Sciences*, 276(1669):2963–2969, Aug. 2009. doi: 10.1098/rspb.2009.0500. URL https://royalsocietypublishing.org/doi/full/10.1098/rspb.2009.0500. Publisher: Royal Society.

T. Philippi. Bet-Hedging Germination of Desert Annuals: Variation Among Populations and Maternal Effects in Lepidium lasiocarpum. *The American Naturalist*, 142(3):488–507, Sept. 1993. ISSN 0003-0147. doi: 10.1086/285551. URL https://www.journals.uchicago.edu/doi/abs/10.1086/285551. Publisher: The University of Chicago Press.

T. Philippi and J. Seger. Hedging one's evolutionary bets, revisited. *Trends in Ecology & Evolution*, 4(2):41–44, Feb. 1989. ISSN 01695347. doi: 10.1016/0169-5347(89)90138-9. URL https://linkinghub.elsevier.com/retrieve/pii/0169534789901389.

T. Pinceel, F. Buschke, A. Geerts, J. Vanoverbeke, L. Brendonck, and B. Vanschoenwinkel. An empirical confirmation of diversified bet hedging as a survival strategy in unpredictably varying environments. *Ecology*, 102(11):e03496, 2021. ISSN 1939-9170. doi: 10.1002/ecy.3496. URL https://onlinelibrary.wiley.com/doi/abs/10.1002/ecy.3496. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ecy.3496.

Y. Raynes, C. S. Wylie, P. D. Sniegowski, and D. M. Weinreich. Sign of selection on mutation rate modifiers depends on population size. *Proceedings of the National Academy of Sciences*, 115(13): 3422–3427, Mar. 2018. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1715996115. URL https://www.pnas.org/content/115/13/3422. Publisher: National Academy of Sciences Section: Biological Sciences.

Y. Raynes, C. L. Burch, and D. M. Weinreich. When good mutations go bad: how population size can change the direction of natural selection. Technical report, bioRxiv, Apr. 2021. URL https://www.biorxiv.org/content/10.1101/2021.04.19.440541v1. Section: New Results Type: article.

M. Richard and G. Yvert. How does evolution tune biological noise? *Frontiers in Genetics*, 5, 2014. ISSN 1664-8021. URL https://www.frontiersin.org/articles/10.3389/fgene.2014.00374.

C. D. Schlichting and M. Pigliucci. Phenotypic evolution: a reaction norm perspective. *Phenotypic evolution: a reaction norm perspective.*, 1998. URL https://www.cabdirect.org/cabdirect/abstract/19980108896. Publisher: Sinauer Associates Incorporated.

J. P. Scholl, L. Calle, N. Miller, and D. L. Venable. Offspring polymorphism and bet hedging: a large-scale, phylogenetic analysis. *Ecology Letters*, 23(8):1223–1231, 2020. ISSN 1461-0248. doi: 10.1111/ele.13522. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/ele.13522. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ele.13522.

A. M. Simons. Fluctuating natural selection accounts for the evolution of diversification bet hedging. *Proceedings of the Royal Society B: Biological Sciences*, 276(1664):1987–1992, June 2009. doi: 10.1098/rspb.2008.1920. URL https://royalsocietypublishing.org/doi/full/10.1098/rspb.2008.1920. Publisher: Royal Society.

A. M. Simons. Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B: Biological Sciences*, 278(1712):1601–1609, June 2011. doi: 10.1098/rspb.2011.0176. URL https://royalsocietypublishing.org/doi/full/10.1098/rspb.2011.0176. Publisher: Royal Society.

M. Slatkin. Hedging one's evolutionary bets. *Nature*, 250(5469):704–705, Aug. 1974. ISSN 1476-4687. doi: 10.1038/250704b0. URL https://www.nature.com/articles/250704b0. Number: 5469 Publisher: Nature Publishing Group.

J. Starrfelt and H. Kokko. Bet-hedging–a triple trade-off between means, variances and correlations. *Biological Reviews of the Cambridge Philosophical Society*, 87(3):742–755, Aug. 2012. ISSN 1469-185X. doi: 10.1111/j.1469-185X.2012.00225.x.

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, and J. Michiels. Frequency of antibiotic application drives rapid evolutionary adaptation of Escherichia coli persistence. *Nature Microbiology*, 1(5):1–7, Mar. 2016. ISSN 2058-5276. doi: 10.1038/nmicrobiol.2016.20. URL https://www.nature.com/articles/nmicrobiol201620. Number: 5 Publisher: Nature Publishing Group.

D. L. Venable. The Evolutionary Ecology of Seed Heteromorphism. *The American Naturalist*, 126 (5):577–595, Nov. 1985. ISSN 0003-0147. doi: 10.1086/284440. URL https://www.journals.uchicago.edu/doi/10.1086/284440. Publisher: The University of Chicago Press.

D. L. Venable. Bet hedging in a guild of desert annuals. *Ecology*, 88(%), May 2007. doi: https://doi.org/10.1890/06-1495.

H. I. Weller, H. López-Fernández, C. D. McMahan, and E. L. Brainerd. Relaxed Feeding Constraints Facilitate the Evolution of Mouthbrooding in Neotropical Cichlids. *The American Naturalist*, 199(6):E197–E210, June 2022. ISSN 0003-0147, 1537-5323. doi: 10.1086/719235. URL https://www.journals.uchicago.edu/doi/10.1086/719235.

A. O. B. Whitlock, K. M. Peck, R. B. R. Azevedo, and C. L. Burch. An Evolving Genetic Architecture Interacts with Hill–Robertson Interference to Determine the Benefit of Sex. *Genetics*, 203(2):923–936, June 2016. ISSN 0016-6731, 1943-2631. doi: 10.1534/genetics.116.186916. URL https://www.genetics.org/content/203/2/923. Publisher: Genetics Section: Investigations.

Y. Yasui. Evolutionary bet-hedging reconsidered: What is the mean–variance trade-off of fitness? *Ecological Research*, 37(3):406–420, 2022. ISSN 1440-1703. doi: 10.1111/1440-1703.12303. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/1440-1703.12303. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/1440-1703.12303.

Yumpu.com. R: A Language and Environment for Statistical Computing, May 2010. URL https://www.yumpu.com/en/document/view/6853895/r-a-language-and-environment-for-statistical-computing.

Supplemental Materials

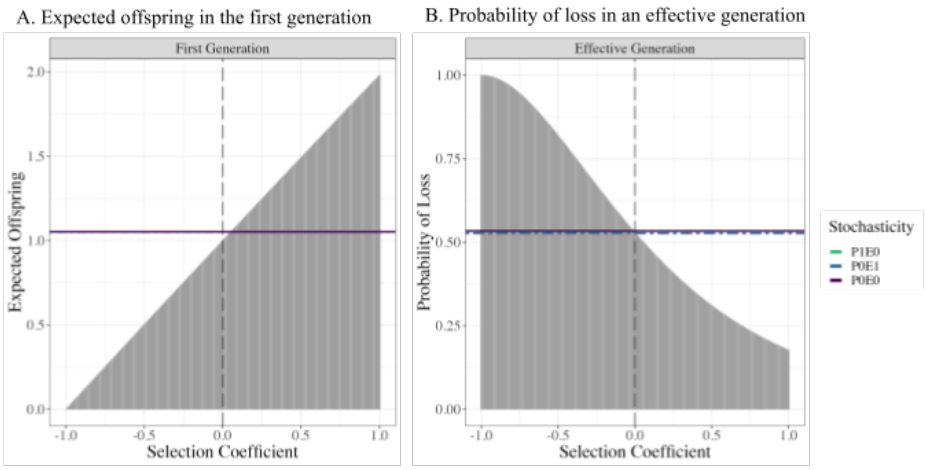

Figure S1: A. When $\Delta GMF = 0$, increasing variance in fitness in a single generation via phenotypic stochasticity causes bet hedging to be less beneficial than expected by increasing probability of loss, but not it does not impact expected offspring counts. In an invariable environment, the relationship between expected offspring and fitness in a single generation is linear. Thus, increasing variance in fitness will have no effect on expected offspring in the first generation. Indeed, we find no difference between the expected offspring counts in a single generation of the P1E0 model compared to P0E0 model. B. When $\Delta GMF = 0$, increasing variance in fitness across an effective generation via environmental stochasticity causes bet hedging to be more beneficial than expected by increasing expected offspring, not through probability of loss. In an invariable environment, the relationship between probability of loss and fitness across an effective generation is neither completely linear nor completely concave up. However, for the selection coefficients experienced in our model, the relationship is largely linear. Thus, increasing variance in fitness is expected to have little to no effect on expected offspring in the first generation. Indeed, we find that the probability of loss for the P0E1 model is only slightly less than that of the P0E0 model.

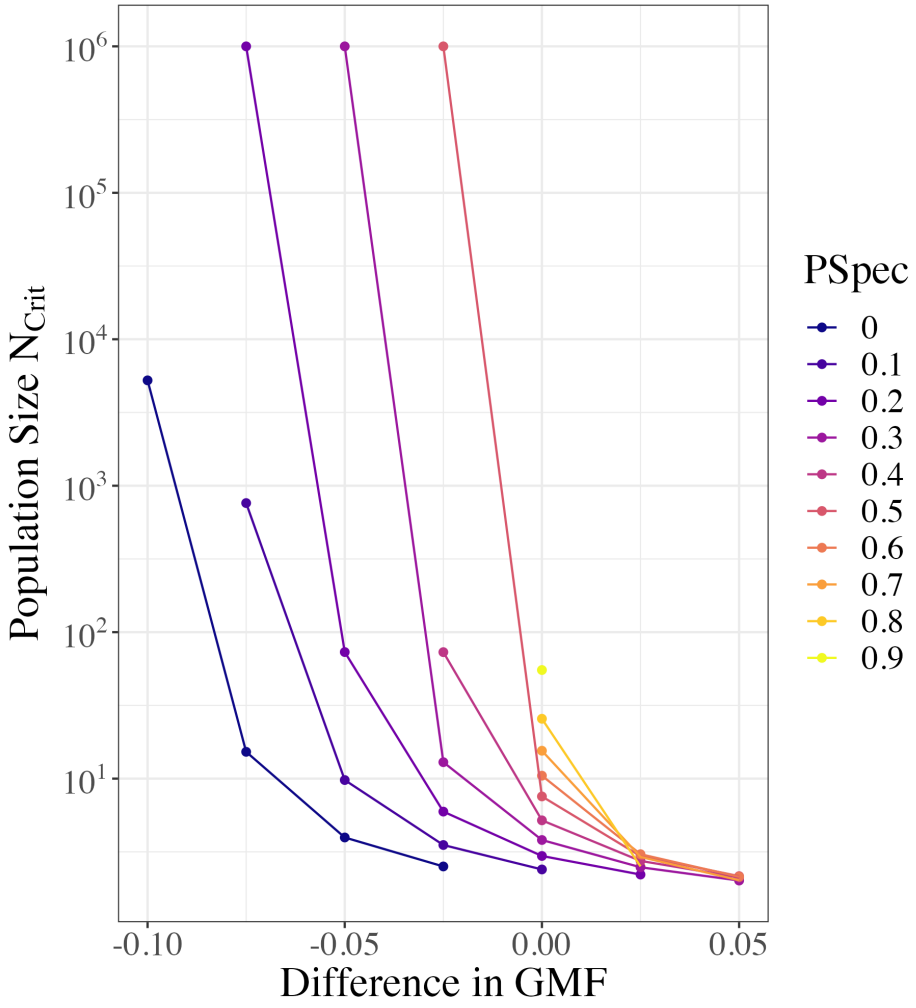

Figure S2: We show the relationship between N_{Crit}, or the population size where the NP_{fix} curve crosses the neutral benchmark before becoming beneficial, and ΔGMF for points that exhibit sign inversion in the P1E1 model. As the GMF of the bet hedger decreases, N_{Crit} increases logarithmically. N_{Crit} depends not only on ΔGMF, but also P_{Spec}.