Adaptive Significance of Amphicarpy as a Bet-hedging Strategy in American Hog-peanut

Brown University
Honors Thesis in Computational Biology

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April 20, 2024

Abstract

Environmental instabilities call for organisms to develop adaptations for long-term survival. Bet-hedging is one such strategy aimed at reducing the risk of extinction in changing environments, where a population sacrifices its short-term mean fitness in order to reduce variance of fitness in the long run. One potential bet-hedging strategy in plants is amphicarpy, a reproductive behavior where individuals produce two morphologically distinct types of flowers and seeds: subterranean and aerial. Subterranean seeds are produced asexually and are large, robust, and fecund when mature. On the other hand, aerial seeds are less fit than their underground counterparts but are more abundant, occasionally result from sexual reproduction, and experience delayed germination. It has been hypothesized that amphicarpy is a bet-hedging strategy, however rigorous tests of whether and when amphicarpy qualifies as adaptive bet-hedging are lacking. I evaluated amphicarpy as a form of bet-hedging in the American hog-peanut (*Amphicarpaea bracteata*), an amphicarpic annual native to a wide range of environments. I simulated the evolution of amphicarpy using a stochastic Wright-Fisher model incorporating a seed bank (delayed germination). Empirical parameters were drawn from published field studies and my experiments on American hog-peanut plants grown in the university greenhouse. Results showed that amphicarpy in American hog-peanut is an adaptive bet-hedging strategy in a variable environment, and displayed several trends in the parameter space driving the adaptiveness of amphicarpy.

Keywords: bet-hedging, annual plants, mating systems, seed bank, amphicarpy

Acknowledgements

I would like to thank Dr. Daniel Weinreich, Dr. Maya Weissman, and David Peede for their generous guidance and support during this project as well as through the past two years. I'm extremely grateful for how they have, as scientists, mentors, and friends, influenced me and shaped my undergraduate experience into a memorable one.

I would like to thank Dr. Rebecca Kartzinel for being the second reader of this thesis and providing the greenhouse space that made the experiments possible, as well as her 2016 work which was an inspiration behind Team Hog-peanut. I would also like to thank greenhouse managers Nick and Sherry who kindly watered the plants when I occasionally forgot to.

I would like to thank Maria Cardone for kindly helping me with posters and bearing with my untimely email replies, as well as Sabrina Boyk and everyone else in the CCMB for making it an enjoyable workplace with their positive energy.

I would like to thank my friends Chris Shin ('24) for providing me with insights and suggestions, as well as Joanne Chen (RISD '25) for making the illustration.

I would like to thank Dr. Will Fairbrother and everyone in the Fairbrother Lab for the invaluable experience and knowledge I acquired during my time in the lab. I would also like to thank Jenn Vieira from Brown Technology Innovations for asking how my days are during meetings.

Lastly, I would like to thank BrownConnect for the UTRA award back in the summer of 2022 which enabled me to start my undergraduate research with the Weinreich Lab and the EEOB department. I would also like to thank the Maria L. Caleel '87 Memorial Undergraduate Biology Research Fellowship for the prestigious honor and generously funding this project.

Contents

1 Introduction

1.1 Evolutionary bet-hedging

Why do we see some phenotypes more than others in a population? The theory of natural selection is widely accepted to explain the divergence of traits: those that are beneficial to survival and reproduction rise in frequency in a population, while others eventually go extinct. When environments are constant over time, we expect to see traits adapted to those conditions evolve. However, when environments are not constant and when they change rapidly and unpredictably, traits that are beneficial in one generation can become deleterious in the next. Under those conditions, directional selection for the short-term fittest trait may no longer be adaptive. Thus, organisms must rely on alternative strategies that can ensure survival.

Evolutionary bet-hedging is one such strategy. Coined from a term used in investment and finance, bet-hedging can be viewed as a risk-preventing strategy with the implication of the market being unpredictable. In a volatile market, stocks that were good one week can be bad the next; as such, novice investors may generally be advised to diversify their portfolio, or to invest in low-risk options even if it means low returns. Similarly, in the setting of evolutionary biology, the theory of bet-hedging posits that avoiding high-risk, high-reward traits can be adaptive, even if it comes at the expense of lowering a population's short-term payout, or fitness. Mathematically, bet-hedging is a strategy that sacrifices the short-term arithmetic mean fitness in order to decrease the variance of fitness (Simons (2011), Slatkin (1973), Philippi and Seger (1989)). In other words, evolutionary bet-hedging presents a trade-off between the mean and the variance of fitness among a population in order to combat environmental variability (Starrfelt and Kokko (2012)).

The majority of bet-hedgers observed in nature utilize the diversified bet-hedging strategy, exemplifying the phrase *don't put all your eggs in one basket* (Simons (2011)). Take, for instance, an organism that produces phenotypically diverse offspring, such that some are well-adapted in some environments, and others are better adapted under different conditions. This reproductive pattern means that fewer offspring will be well-adapted each generation, lowering the population mean fitness in the short term due to the lower number of individuals with the highest fitness. However, as environmental conditions vary, the ill-adapted phenotypes will each experience favorable environments in which their fitness advantage emerges. If the variation in the environment is frequent and strong enough, the primary phenotype may face extinction, while those phenotypes with alternative reproductive strategies will be able to rescue the population.

While bet-hedging has been observed across the tree of life, from bacteria (Balaban et al. (2004)) to frogs (Crump (1981)), one of the best studied taxa is annual plants (Childs et al. (2010), Simons (2011)). Delayed germination is one common bet-hedging strategy observed in annual plants. Some annuals may germinate only a portion of their seeds produced in a given year, which is a form of diversified bet-hedging. Seeds that delay germination are stored in the soil in an abstracted "seed bank", and can be dormant for many years (or generations). Delayed seed germination lowers short term fitness, as fewer active offspring are produced. But it also lowers risk in case of a severe drought: if all seedlings die, the seeds in the seed bank have the chance to break dormancy and rescue the dying population. Thus, seed banking prevents unfavorable environmental conditions, such as drought and insufficient sunlight, from wiping out the entire population. For example, to combat occurrences of extreme drought, desert annuals such as Spring Pygmycudweed (*Evax multicaulis*) germinate below 10% of their seeds, while only 50% of the seeds germinate in most other dessert annuals (Venable (2007)). Interestingly, delayed germination can also occur as a part of another reproductive behavior: amphicarpy.

1.2 Amphicarpy and American hog-peanuts

Amphicarpy is characterized by the production of two distinct types of flowers on the same plant: aerial and subterranean (Zhang et al. (2020)). The aerial flowers are borne above the ground, producing small seeds in vast numbers. Aerial seeds engage in delayed seed germination, with some remaining dormant for years underground, providing a backup seed bank in times of drought. The small size of the seeds and their positionality in the air allow for some degree of seed dispersal, contrary to their subterranean counterpart fixed to the roots. Subterranean flowers are found underground, and produce a few large seeds on each plant. The subterranean phenotype has a higher fitness than their aerial counterparts, due to the large size of both the seeds and the adult plants that arrive from them. This dual reproductive strategy is reminiscent of diversifying bet-hedging, as producing both seed types allows plants to mitigate risks: the abundance of aerial seeds as well as their robust seed bank may serve to rescue the population during a drought, in which the normally fit subterranean seeds may not survive.

Amphicarpy has been observed in 67 herbaceous species across 39 genera and 13 families of angiosperms, including the American hog-peanut (Zhang et al. (2020)). The American hog-peanut (*Amphicarpaea bracteata*) is a herbaceous perennial vine native to North America, found primarily in moist woodlands, riverbanks, and floodplains across eastern and central regions of the continent. Aside from droughts, the hog-peanuts can also face predation from birds and hogs, compromising the survivorship of its large and nutritious subterranean seeds. It follows that another benefit of aerial seeds in this case could be a reduced chance of extinction due to predation.

1.3 Gap in knowledge and objectives

It has been hypothesized that amphicarpy is a bet-hedging strategy, however rigorous tests of whether or not amphicarpy is adaptive and meets the criteria for bet-hedging are lacking (Zhang et al. (2020)). As climate change induced by global warming causes environments to become more variable, understanding how native species adapt can help inform conservation. As such, studying the evolution of amphicarpy as a bet-hedging strategy can inform both the evolutionary biology behind the amphicarpy phenomenon and how amphicarpic plants respond to climate change.

In this study, I combined greenhouse experiments and population genetics modeling to evaluate whether, and under which circumstances, amphicarpy in the American hog-peanut can be considered adaptive bet-hedging. I simulated the evolution of amphicarpy using stochastic, individual-based Wright-Fisher models incorporating a seed bank. Empirical parameters were drawn from published field study as well as my own experiments conducted on American hog-peanut plants grown in the Brown University Plant Environmental Center. Through models, I found that amphicarpy in American hog-peanut is an adaptive bet-hedging strategy in more variable environments, and identified several trends in the parameter space driving the adaptiveness of amphicarpy. My results shed new light on the adaptive significance of amphicarpy as bet hedging, providing predictions for future research in the field.

2 Methods

2.1 Model construction

I constructed a stochastic, individual based model in `Julia` which simulates the establishment of a single invading mutant in a resident population. The model assumes Wright-Fisher conditions (diploid, selfing population with non-overlapping generations) except allowing for extinction. The model probablistically determines the number of offspring based on the fitness of the adult phenotype. I allow for hard-selection, meaning that the population size can change each generation, but must remain below a certain carrying capacity ($K = N$).

Populations are characterized by the ratio between subterranean seeds and aerial seeds, hereafter termed the "amphicarpic ratio". The amphicarpic ratio is parameterized using the proportion of subterranean seeds to all seeds produced, P_{sub}. Since chasmogamous aerial flowers are only found during favorable environmental conditions on large plants in the Greenhouse, I assume clonal reproduction (Kartzinel et al. (2016)). In each population, I track active aerial seeds, dormant aerial seeds (stored in the seed bank), and active subterranean seeds separately. I also track whether adults arise from aerial seeds and subterranean seeds (hereafter denoted as "aerial adults" and "subterranean adults"), as the two types of adults differ in fitness.

There are three stages in a reproductive cycle (Figure 1). In the beginning of each generation, each population starts in the `Growth()` stage, in which germinated aerial and subterranean seeds from the previous generation mature into adults. If the sum of the number of realized adults from both populations exceeds the carrying capacity, adults are down-sampled using a multinomial distribution to ensure that adult plants do not exceed the carrying capacity before producing seeds. To simulate environmental variability, in each generation the model randomly determines whether there will be a `drought`, with probability $P_{drought}$, or if the environment will be `normal`, with probability $1-P_{drought}$. During a `drought`, aerial and subterranean adults in both populations die out, leaving only dormant seeds in the bank. When environmental conditions are normal, we utilize fitness measurements that combine survivorship and fecundity to estimate the expected number of offspring in the next generation.

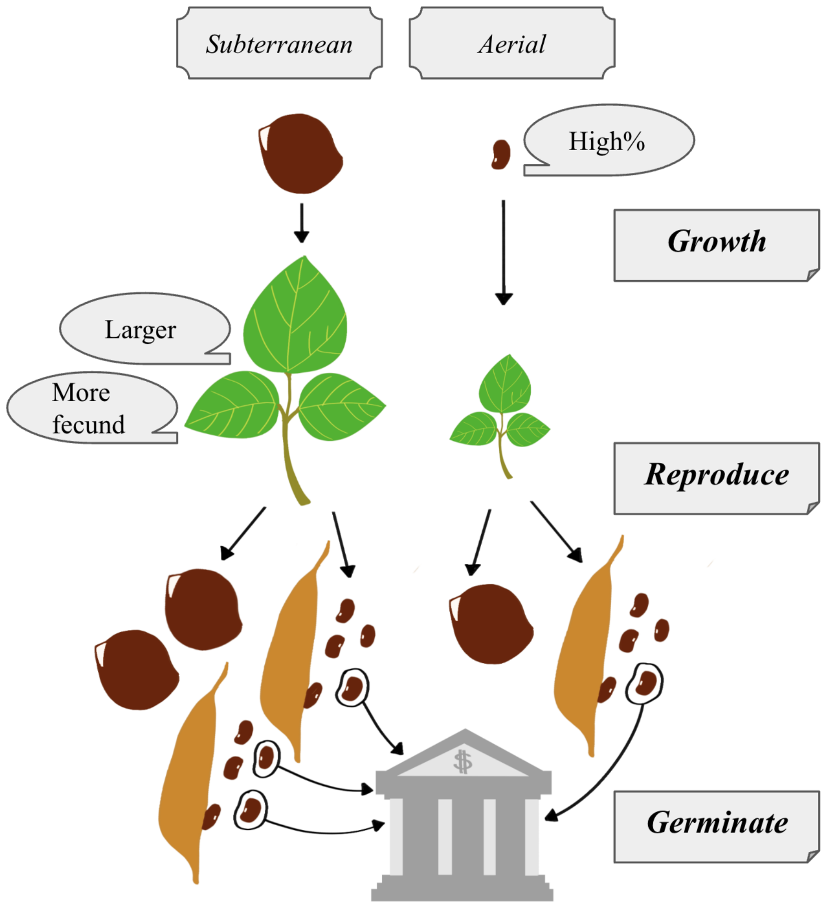
Next, in the `Reproduce()` stage, adults produce seeds according to their fitness (w).

Seeds are partitioned into the two seed types, aerial and subterranean, according to the two populations' amphicarpic ratios, $P_{sub^{Invading}}$ and $P_{sub^{Resident}}$, respectively.

Lastly, seeds undergo the `Germinate()` stage to determine how many seeds will be active in the next generation and how many will enter the seed bank. Aerial seeds produced in the current generation are joined by all dormant seeds in the seed bank. Aerial seeds germinate with probability $P_{germ^{aer}}$, with the remaining seeds staying dormant. Given a maximum number of generations, m, that an aerial seed can stay dormant for in the seed bank before degradation (death), the dormant seeds from the m-th (earliest) generation are dropped and those from the current generation are then deposited into the latest generation of the seed bank. I assume that the probability of dormant seeds germinating does not depend on how long the seeds have been in the bank; in other words, dormant seeds are treated as identical across the seed bank. Subterranean seeds do not engage in delayed seed germination; thus, subterranean seeds germinate with probability $P_{germ^{sub}}$, with the assumption that seeds that fail to germinate die immediately.

For all simulations, the population size and carrying capacity take on values [10, 100, 1000] to reflect the dynamics in populations of different sizes. Simulations were repeated for a total of 10^6 replicates to ensure that a neutral mutation is expected to reach fixation at least 1000 times in a population of 1000. In each replicate, the simulation runs for a maximum of 10^6 generations, or until one of the following scenarios occurs: 1) the `Invading` population reaches fixation, signaled by a sum of zero seeds and adults in the `Resident` population and the sum of adults in the `Invading` population at K; 2) the `Invading` population is extinct, signaled by a sum of zero seeds and adults in the `Invading` population and the sum of adults in the `Resident` population at K; and 3) both populations are extinct, signaled by a sum of of zero seeds and adults in both populations. If none of the above three scenarios occur during the 10^6 generations, the simulation terminates with both populations survived. Fixation outcomes for the `Invading` population respective to the four scenarios,

Figure 1: Model Flow Diagram



Three stages of the reproductive cycle in the model.

`Fixation`, `Loss`, `Mutual Extinction`, and `Mutual Survival`, are tallied across the 10^6 replicates. Probability of fixation of the `Invading` population is normalized by the sum of `Fixation` and `Loss` events as the metric for measuring the adaptiveness of the `Invading` population:

$$NP_{Fixation} = \frac{\sum_{r=1}^{10^6} Fixation}{\sum_{r=1}^{10^6} Fixation + \sum_{r=1}^{10^6} Loss} * N$$

for replicate indexed $r <= 10^6$. Expectation of $NP_{Fixation}$ for a neutral allele is $\frac{1}{N} * N = 1$. Thus, if $NP_{Fixation} > 1$, the trait reaches fixation more frequently than a neutral mutation would, and is considered beneficial; if $NP_{Fixation} < 1$, the trait is considered deleterious.

2.2 Model parameterization

To parameterize the model, I acquired estimations of germination rates of aerial and subterranean seeds and fecundity for aerial and subterranean adults. For some simulations, I utilized published fitness measurements from field experiments in Joseph Trapp and Hendrix (1988).

The average chance of drought was estimated using rainfall data in the past 50 years (Figure S1). I used a $P_{drought} = 0.05$ to represent a the probability of drought in an average year and $P_{drought} = 0.1$ to represent a year with more severe drought conditions.

2.2.1 Greenhouse experiments

I also conducted germination assays under optimal growing conditions in the Brown University Plant Environmental Center (hereafter referred to as the Greenhouse) which housed at most around 200 American hog-peanut plants at one time. American hog-peanut aerial seeds were originally collected from Lincoln, Rhode Island (LR), Collin County, Texas (LG), and Kankakee, Illinois (KM). For each locality I sampled 50 aerial seeds from four maternal lines, for a total of 200 aerial seeds per locality. To break physical dormancy, I first mechanically scarified every seed by nicking the seed coat with a razor blade, allowing moisture to reach the embryo. After scarification, for every locality, all the seeds were planted individually in a 200-cell tray. To break physiological dormancy, I cold-stratified each of the three trays of seeds for 60 days. Afterwards, trays were placed in controlled temperature and allowed to germinate with watering as needed. Germination statuses were recorded every morning from September to October 2023. Among the three trays of a total of 600 aerial seeds produced by plants originating from three geographical locations, germination rates were 71%, 71.5%, and 97.5% , respectively for the three lines (Figure S2).

3 Results

3.1 Fitness parameters

Fitness parameters collected from both literature and greenhouse experiments were utilized in various rounds of simulations (Table 1). The germination rates estimated from Greenhouse experiments are much larger than field measurements from Joseph Trapp and Hendrix (1988), due to the drastically different growing environments.

Table 1: American hog-peanut fitness parameters and sources

Source	Trapp and Hendrix				Greenhouse			
Phenotype	Aerial		Subterranean		Aerial		Subterranean	
	Seed	Adult	Seed	Adult	Seed	Adult	Seed	Adult
%Germination	6.5	-	86.1	-	70*	-	100*	-
Fecundity[1]	-	11.2	-	151.39	-	-	-	-
%Survivorship[2]	-	100	-	100	-	100	-	100
Relative fitness[3]	-	-	-	-	-	?	-	1

*Parameter values marked with * are estimated from germination assays in the Greenhouse.*
[1] *Fecundity is the mean of the sum of aerial and subterranean seeds produced by all adults measured.*
[2] *Survivorship is assumed to be 100%.*
[3] *Relative fitness of subterranean adults is set to 1, for a parameter space survey on the relative fitness of aerial adults.*

Germination assays conducted in the greenhouse (Figure S2) informed the estimation on aerial seed germination rate (Table 2). Aerial germination rates ranged from 50% to 100%, with the KM line significantly out-germinating the other two at an average of 97.5%. I took the median of mean by locations and approximated it to 70% for trend analyses, representing the average germination rate of aerial seeds under ideal growing conditions (Figure S2). Although subterranean germination was not assayed, germination of these large seeds in the Greenhouse has always been robust and therefore was approximated to 100% for trend analyses.

Table 2: Germination rates of aerial seeds produced and grown in the Greenhouse

Line[1]	LR				LG				KM			
ID[2]	12H1B	12H4B	12H5A	12H6A	23	25	26	28	1	2	3	4
%Germination	94	72	68	50	84	60	72	70	92	98	100	100

[1] *Each line represents a locality where the original seeds were collected. LR: Lincoln, Rhode Island; LG: Collin County, Texas; KM: Kankakee, Illinois.*
[2] *Each ID represents a subterranean mother plant from the respective line. 50 aerial seeds were randomly sampled from all the aerial seeds collected from the respective mother plant.*

3.2 Simulations

In order to determine when amphicarpy is adaptive, I simulated the evolution of amphicarpy using a stochastic, individual-based Wright-Fisher model. I used $NP_{Fixation}$, the normalized probability of fixation for the `Invading` population, to assay whether a trait is adaptive (Spirito et al. (1993), Neher and Shraiman (2011), Raynes et al. (2018)). $NP_{Fixation}$ compares the probability of fixation to that of neutral mutation. At $NP_{Fixation} > 1$, the invading trait is considered beneficial, as it reaches fixation more frequently than neutral; at $NP_{Fixation} < 1$, the invading trait is considered deleterious.

3.2.1 Surveying the amphicarpic ratio with fecundity as a fitness approximation

First, I used published parameters from field measurements in natural conditions from Joseph Trapp and Hendrix (1988) to simulate the evolution of amphicarpy. Each P_{sub} takes values on both a coarse scale with range $[0, 1]$ in intervals of 0.25 and a fine scale with range $[0, 0.1]$ in intervals of 0.025. I surveyed across the range of $P_{sub^{Invading}}$ and $P_{sub^{Resident}}$ and recorded $NP_{Fixation}$ for each combination.

With no environmental variability ($P_{drought} = 0$), the optimal seeding strategy for the `Invading` population is to produce as many subterranean seeds as possible, or $P_{sub^{Invading}} = 1$ (monocarpy of subterranean seeds) (Figure 2a). At $P_{sub^{Invading}} = 1$, the mutant is always beneficial, regardless of the resident strategy; conversely, when $P_{sub^{Resident}} = 1$, all possible $P_{sub^{Invading}}$ are deleterious. This is because the fitness of the subterranean adults is a magnitude above that of the aerial adults, approximated with their respective fecundity.

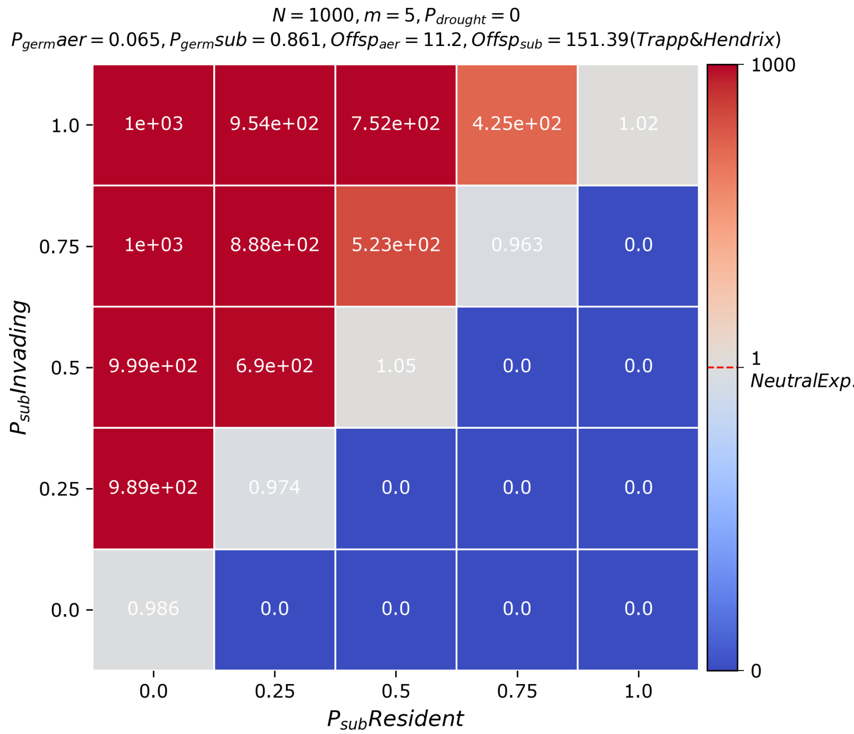
When there is a non-zero probability of `drought`, amphicarpy in the `Invading` population can be beneficial (Figure 2b). For instance, when $P_{sub^{Resident}} = 0$, the invading strategy that yields the highest probability of fixation is $P_{sub^{Invading}} = 0.5$. At $P_{sub^{Resident}} \neq 1$, diversified amphicarpy can be adaptive, however higher values of $P_{sub^{Invading}}$ are more adaptive in general. Interestingly, when $P_{sub^{Resident}} = 1$, any amount of bet-hedging is beneficial. This supports the hypothesis that amphicarpy is more advantageous than monocarpy in changing environments. However, the resulting range of beneficial $P_{sub^{Invading}}$ values is significantly higher and broader than the observed P_{sub} in nature ($<= 5\%$) (Figure 2). This is likely due to the overwhelming fitness advantage brought by the 10-fold higher fecundity of the subterranean adults used in the model to approximate fitness. The overpowering fitness advantage is also reflected in the extremely high $NP_{Fixation}$ values.

Given these results, my model using the Joseph Trapp and Hendrix (1988) fitness estimates cannot explain the evolution of amphicarpy, specifically the observed amphicarpic ratio, in American hog-peanuts. This suggests that the model may not have captured some benefits of the aerial seeds.

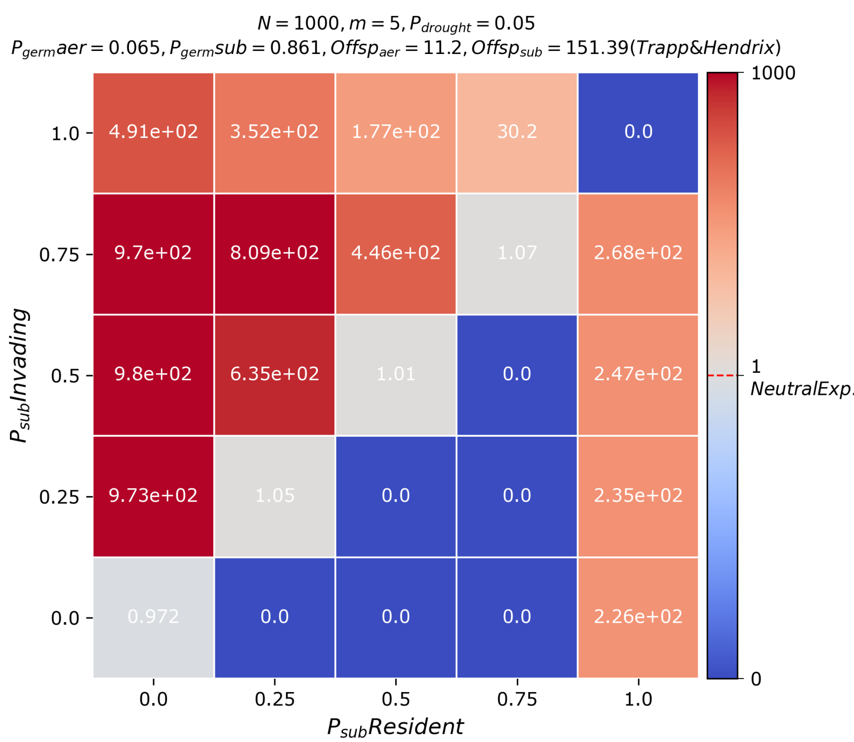
3.2.2 Surveying the amphicarpic ratio within a range of relative fitness values

As such, I employed an alternate approach to determine the range of fitness values where the degree of bet-hedging observed in nature is beneficial. I ran the simulation with the same P_{germ} values from Joseph Trapp and Hendrix (1988) but across a range of relative fitness assigned to the aerial phenotype. Treating monocarpy of subterranean seeds as the dominant resident seeding strategy, I set w_{sub} to 1 and w_{aer} to values ranging from 0.5 to 2.0 in intervals of 0.1, and then surveyed $P_{sub^{Invading}}$ and $P_{sub^{Invading}}$ in the same fashion as the previous experiment.

Figure 2: Normalized probability of fixation for every combination of amphicarpic ratios



(a) *In a population of 1000 and a default seed bank length of 5 generations, in a stable environment, the Invading population's optimal strategy is monocarpy of subterranean seeds.*



(b) *Under the same configuration with a probability of drought, amphicarpy in the Invading population can be more beneficial than monocarpy.*

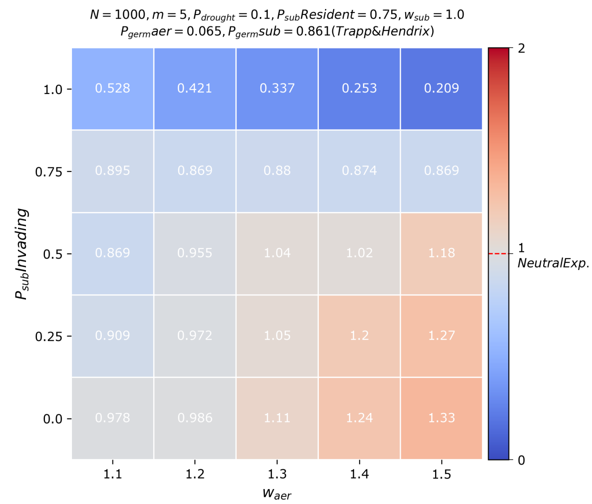
In a population of 1000 adults, at $w_{aer} < 1.3$, the simulation produced trends similar to those observed in the previous experiment: at $P_{drought} \neq 0$ and $P_{sub^{Resident}} \neq 1$, higher values of $P_{sub^{Invading}}$ are more adaptive (Figure S3a). However, at $w_{aer} >= 1.3$, amphicarpy can be beneficial even when $P_{sub^{Invading}} < P_{sub^{Resident}}$ (Figure 3a, S3b). Although this $w_a er$ threshold fluctuates with population size and the severity of drought (Figure 3c,d), the trend persists across populations of all sizes (Figure S4). This new trend starts to align with the low P_{sub} observed in nature.

Nonetheless, a true approximation of w_{aer} should always be below $w_{sub} = 1$ due to the empirically lower fitness of the aerial phenotype which fundamentally constitutes this bet-hedging behavior. I then ran the same simulations with germination rates estimated from the Greenhouse assays, much higher than those recorded in Joseph Trapp and Hendrix (1988). Here, in a population of 1000 adults, the same w_{aer} threshold where the trend ($NP_{Fixation} > 1$ observed at $P_{sub^{Invading}} < P_{sub^{Resident}}$) starts to occur drops to 0.7 (Figure 3b, S5). A more realistic $w_{aer} = 0.7$ suggests that aerial seed germination rate has a considerable impact on the adaptiveness of amphicarpy.

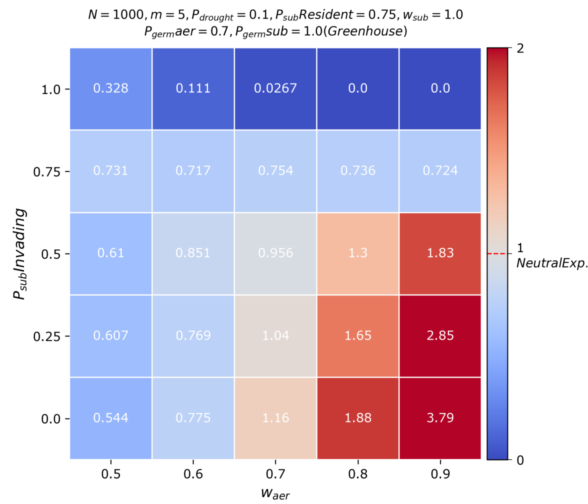
3.2.3 Estimating the optimal range of aerial seed germination rate

Because substituting aerial germination rates resulted in the lower bound of the optimal range of aerial fitness significantly decreasing into an realistic range of below 1.0, I wanted to investigate the sensitivity of amphicarpy success to germination rates. As such, I examined the effect of varying aerial seed germination rate on the adaptiveness of amphicarpy in regions of the parameter space. Given the estimated observation of $P_{sub} <= 5\%$ and the assumption that a high P_{sub} (primarily monocarpy of subterranean seeds) is the resident strategy, I set $P_{sub^{Invading}}$ to 0.05 and the range of $P_{sub^{Resident}}$ values to [0.7, 0.8, 0.9]. Setting $P_{germ^{sub}}$ to 1.0, I ran the simulation with every combination of $P_{sub^{Invading}}$ and $P_{sub^{Resident}}$ and surveyed $P_{germ^{aer}}$ both on a coarse scale of [0.0, 1.0] in intervals of 0.1 and on a fine scale of [0.0, 0.1]

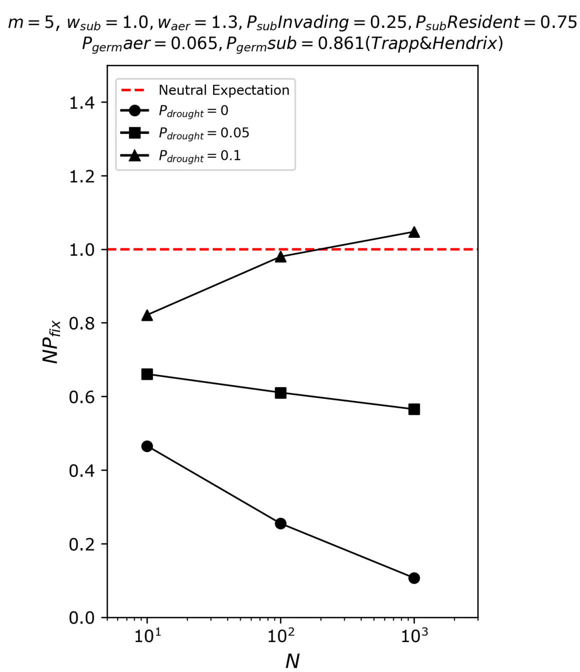
Figure 3: Normalized probability of fixation under different combinations of aerial fitness and amphicarpic ratio in a population of 1000 and a seed bank duration of 5 generations



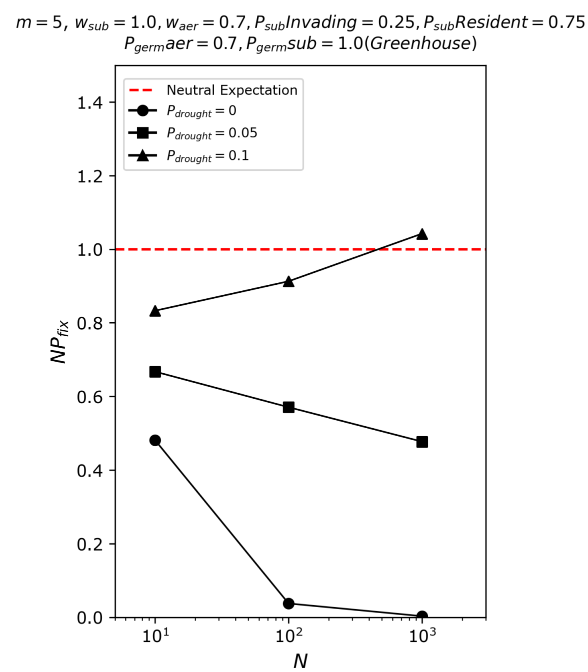
(a) *Using germination rates from field measurements, amphicarpy starts to be beneficial when* $w_{aer} = 1.3$.



(b) *Using germination rates from Greenhouse measurements, amphicarpy starts to be beneficial at a lower* $w_{aer} = 0.7$.



(c) *At* $w_{aer} = 1.3$, *amphicarpy is only beneficial in a large population under severe drought.*



(d) *At* $w_{aer} = 0.7$, *amphicarpy is only beneficial in a large population under severe drought.*

in intervals of 0.01.

In general, across the range of w_{aer} surveyed, amphicarpy is deleterious at low w_{aer} and $P_{germ^{aer}}$ values unless the population experiences extreme drought ($P_{drought} = 0.1$) (Figure S6), once again exemplifying the ability of an aerial seed bank to combat extreme environmental conditions.

Notably, the relationship between $NP_{Fixation}$ and the combined effect of w_{aer} and $P_{germ^{aer}}$ is not linear, contrary to the intuition that the more germinated aerial seeds and the fitter the aerial adults are, the more adaptive the `Invading` population. There is indeed a positive correlation between w_{aer} and $NP_{Fixation}$, suggested by the increasing $NP_{Fixation}$ along the entire range of w_{aer} horizontally (for every value of $P_{germ^{aer}}$) (Figure 4a,b). However, $NP_{Fixation}$ does not always increase with $P_{germ^{aer}}$. Instead of maximizing at 100% germination, $P_{germ^{aer}}$ finds its most universally-beneficial value (i.e. when the widest range of w_{aer} can result in beneficial amphicarpy in combination) at an intermediate (Figure 4a,b). Although the simulations do not always produce a single $P_{germ^{aer}}$ which is the most universally-beneficial due to the granularity of parameters, this value should exist either exactly once or within a very fine range, therefore hereafter referred to as the "threshold". In the case of $N = 1000$ under moderate drought ($P_{drought} = 0.05$), this threshold $P_{germ^{aer}}$ exists at 0.5, where the threshold w_{aer} (the lowest beneficial w_{aer}) is 0.6 (Figure 4a). This trend persists under a severe drought ($P_{drought} = 0.1$), although the threshold $P_{germ^{aer}}$ is reduced significantly to around 0.1 in the same population (Figure 4b).

Interestingly, the threshold $P_{germ^{aer}}$ that allows for the widest beneficial range of w_{aer} does not consistently produce the highest $NP_{Fixation}$ on average. It appears that the $P_{germ^{aer}}$ which maximizes $NP_{Fixation}$ increases with w_{aer} until asymptoting at some intermediate value in the range of $[0, 1]$ (Figure 4c,d).

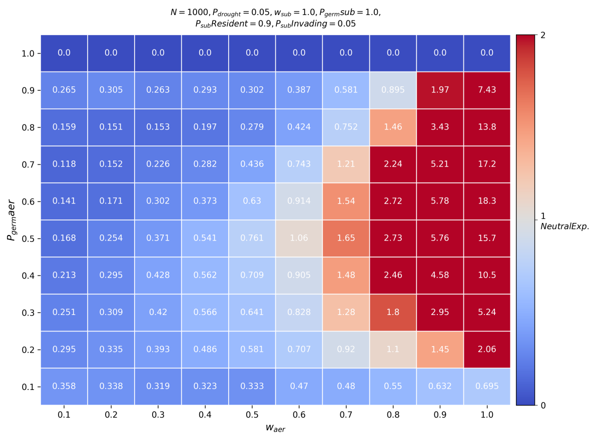
In a follow-up set of experiments where the seed bank length m is variable, the same trend in $P_{germ^{aer}}$ threshold remains (Figure S8a). As m increases (i.e. aerial seeds are allowed to stay in the seed bank for more generations), the threshold $P_{germ^{aer}}$ and w_{aer} both become lower, occupying a larger region in the parameter space where amphicarpy is beneficial (Figure S8b).

Taken together, the germination surveys show that the optimal range of germination rate is not the highest; instead, it often occurs at some intermediate percentage. More specifically, the adaptiveness of aerial seed germination rate in amphicarpy depends on the severity of drought, the fitness of the aerial phenotype relative to that of the subterranean, as well as the length of dormancy (seed bank). The amphicarpic ratio of the resident population and the population size may also skew the adaptiveness of amphicarpy (Figure S6, S7).

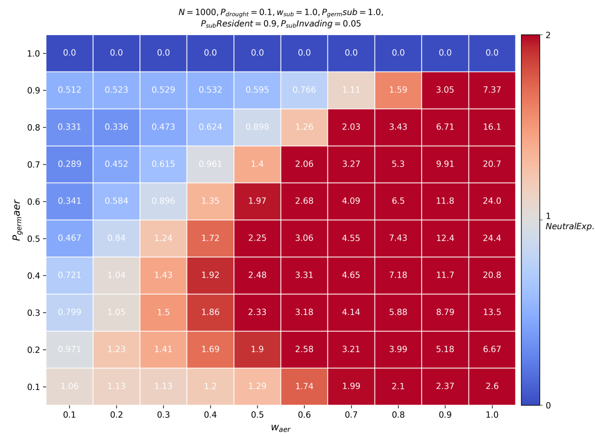
4 Discussion

Amphicarpy is a complex risk-spreading strategy. In American hog-peanuts, not only does it involve diversifying seed and adult phenotypes in morphology (distinct difference in physical size) as well as mode of reproduction (exclusively selfing in subterranean flowers and a mix of selfing and out-crossing in aerial flowers), the trade-off also differs in dormancy (aerial seeds can delay germination while subterranean seeds immediately germinate). While amphicarpy has been hypothesized to be a bet-hedging strategy, no studies have been able to show that the degree of bet-hedging is adapting in American hog-peanuts. Through stochastic simulation of the establishment of a single mutant, informed with published data as well as observations and measurements in the Greenhouse, I was able to show that amphicarpy in American hog-peanut is beneficial under variable environments, and can therefore be considered a form of bet-hedging. While confining some parameters within certain ranges which are observed to be empirical under either a natural or optimal growing condition, the model was able to uncover several notable trends regarding amphicarpy bet-hedging. As the severity of drought, the size of the population, the proportion of subterranean seeds, and the

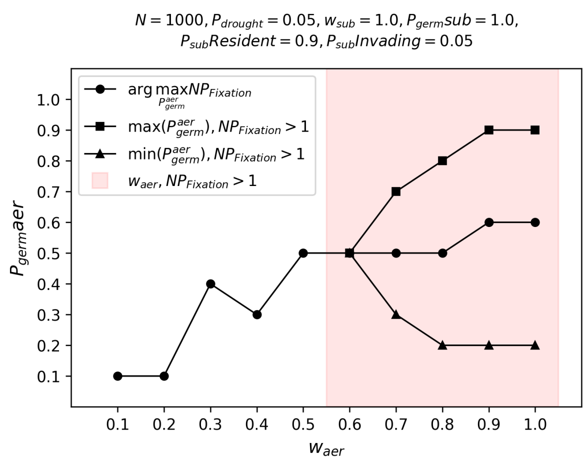
Figure 4: Normalized probability of fixation for every combination of aerial fitness and aerial germination rate in a population of 1000 and a seed bank duration of 5 generations



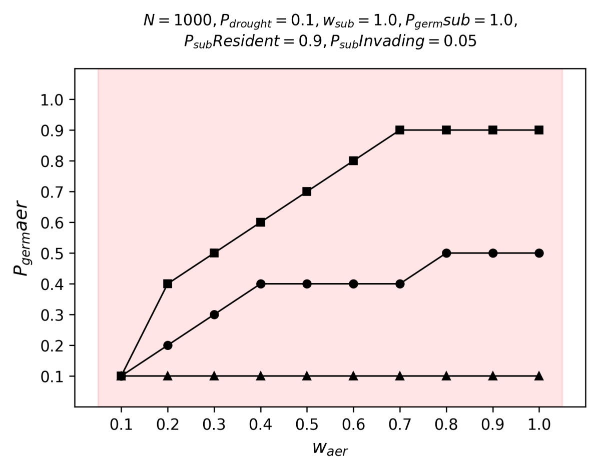
(a) *There exists a non-linear relationship between the combined effect of fitness and germination rate. Under moderate drought, the threshold germination rate which maximizes the range of fitness is 0.6.*



(b) *The trend in (a) persists. Under severe drought, the threshold germination rate occurs at $w_{aer} = 0.1$. The region of the parameter space where amphicarpy is beneficial has been expanded.*



(c) *Regression of the germination rate which maximizes normalized probability of fixation, and its extrema which yields beneficial amphicarpy at each fitness value, corresponding to (a).*



(d) *Regression of the germination rate which maximizes normalized probability of fixation, and its extrema which yields beneficial amphicarpy at each fitness value, corresponding to (b).*

number of generations aerial seeds can stay dormant in the seed bank increase, amphicarpy is generally more adaptive. As the fitness of the aerial seeds increases, however, the highest germination rate of aerial seeds is not necessarily optimal; instead, an intermediate germination rate is most beneficial for the widest range of plausible aerial fitness. These trends generally displayed the adaptive advantage of having a low-fitness phenotype in response to environmental variability.

Several limitations exist in the duration of the project. Firstly, existing field measurements of American hog-peanut fitness parameters such as fecundity, germination rate, and survivorship, as well as the observed amphicarpic ratio, are scarce. The physiology of hog-peanuts in nature–the horizontal spreading of individual plants and entangling between plants, as well as the popping mechanism of releasing aerial seeds from mature pods–make it difficult to separate individuals from one another, let alone measuring. Our Greenhouse experiments, on the other hand, cannot faithfully recreate the natural conditions. The lack of confidence in the parameter values lead to expansive estimations as well as inferences being made about general trends instead of specific range of values.

Secondly, I made several assumptions about American hog-peanuts that simplified the model, while some aspects of amphicarpy may have been lost. Assuming clonal reproduction, the potential fitness advantage brought by the scarce chasmogomous (out-crossing) aerial flowers was omitted; the observed scarcity of chasmogomous aerial flowers in the Greenhouse may have resulted from a low selective pressure, under which inbreeding had little consequence. Using a seed bank model with uniform probability of seeds breaking dormancy blind to the generation when they were produced may be an approximation that does not reflect the natural biochemical and thermodynamic processes within the seeds. In a model lacking spatial structures, the ability–although limited by the near-ground height of the plants in nature–of aerial seeds to be dispersed by wind or predators may have been overlooked, since the potential to establish a new population with zero competition may be a source of fitness advantage. Taken together, future work to explore the impact of these complications could help clarify our understanding of adaptive bet-hedging via amphicarpy.

Another limitation is that we do not know the initial (or resident) strategy that amphicarpy evolved from. While I performed some experiments across the full range of possible resident strategies (or amphicarpic ratios on a scale of [0, 1]), for most analyses I assume that monocarpy of subterranean seeds was the ancestral state. This is because subterranean seeds have such high fitness under normal environments. In the future, combining this research with phylogenetic comparisons could help illuminate ancestral strategies, providing addition context.

Overall, my analyses provide insights on the evolutionary history of amphicarpy as a unique reproductive trait in plants. If supplemented by real-time field measurements and weather data, the model can be an aid to predicting the dynamics within a local ecosystem with a significant presence of amphicarpic plants. Furthermore, this project holds implications for broader ecological issues induced by global warming. As environmental variability and extreme weather events become more frequent, severe, and unpredictable due to climate change, inferences made from amphicarpic plant measurements can become increasingly crucial. Ultimately, such knowledge can contribute to more effective conservation and management strategies in the face of ongoing environmental challenges.

References

Balaban, Nathalie Q., Jack Merrin, Remy Chait, Lukasz Kowalik, and Stanislas Leibler (2004) “Bacterial persistence as a phenotypic switch,” *Science (New York, N.Y.)*, 305 (5690), 1622–1625, 10.1126/science.1099390.

Childs, Dylan Z., C. J. E. Metcalf, and Mark Rees (2010) “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, 10.1098/rspb.2010.0707.

Crump, Martha L. (1981) “Variation in Propagule Size as a Function of Environmental Uncertainty for Tree Frogs,” *The American Naturalist*, 117 (5), 724–737, 10.1086/283755, Publisher: The University of Chicago Press.

Joseph Trapp, E. and Stephen D. Hendrix (1988) “Consequences of a mixed reproductive system in the hog peanut, Amphicarpaea bracteata, (Fabaceae),” *Oecologia*, 75 (2), 285–290, 10.1007/BF00378611.

Kartzinel, Rebecca Y., Daniel Spalink, Donald M. Waller, and Thomas J. Givnish (2016) “Divergence and isolation of cryptic sympatric taxa within the annual legume Amphicarpaea bracteata,” *Ecology and Evolution*, 6 (10), 3367–3379, 10.1002/ece3.2134, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ece3.2134.

Neher, R A and B I Shraiman (2011) “Genetic Draft and Quasi-Neutrality in Large Facultatively Sexual Populations,” *Genetics*, 188 (4), 975–996, 10.1534/genetics.111.128876.

Philippi, T. and J. Seger (1989) “Hedging one’s evolutionary bets, revisited,” *Trends in Ecology & Evolution*, 4 (2), 41–44, 10.1016/0169-5347(89)90138-9.

Raynes, Yevgeniy, C. Scott Wylie, Paul D. Sniegowski, and Daniel M. Weinreich (2018) “Sign of selection on mutation rate modifiers depends on population size,” *Proceedings of the National Academy of Sciences*, 115 (13), 3422–3427, 10.1073/pnas.1715996115, Publisher: Proceedings of the National Academy of Sciences.

Simons, Andrew M. (2011) “Modes of response to environmental change and the elusive empirical evidence for bet hedging,” *Proceedings. Biological Sciences*, 278 (1712), 1601–1609, 10.1098/rspb.2011.0176.

Slatkin, M. (1973) “Gene flow and selection in a cline,” *Genetics*, 75 (4), 733–756, 10.1093/genetics/75.4.733.

Spirito, F., M. Rizzoni, and C. Rossi (1993) “The establishment of underdominant chromosomal rearrangements in multi-deme systems with local extinction and colonization,” *Theoretical Population Biology*, 44 (1), 80–94, 10.1006/tpbi.1993.1019.

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

Venable, D. Lawrence (2007) "Bet hedging in a guild of desert annuals," *Ecology*, 88 (5), 1086–1090, 10.1890/06-1495.

Zhang, Keliang, Jerry M. Baskin, Carol C. Baskin, Gregory P. Cheplick, Xuejun Yang, and Zhenying Huang (2020) "Amphicarpic plants: definition, ecology, geographic distribution, systematics, life history, evolution and use in agriculture," *Biological Reviews of the Cambridge Philosophical Society*, 95 (5), 1442–1466, 10.1111/brv.12623.

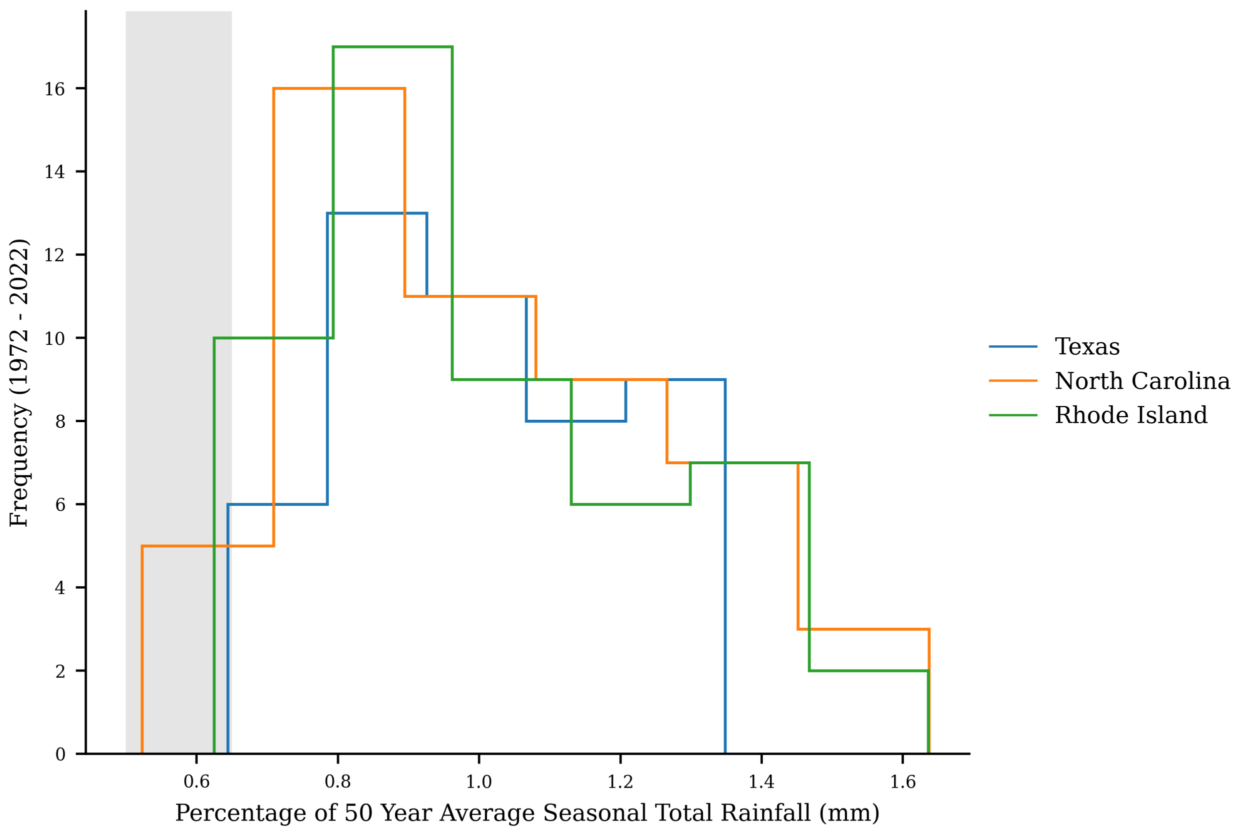
Supplementary Information

Code availability

Scripts and data used to run the experiments and generate the figures and tables can be found at https://github.com/mweissman97/hogpeanut-bethedging/tree/neal.

Supplementary figures

Figure 1: Determining $P_{drought}$ from rainfall data



The average drought percentage in Texas, North Carolina, and Rhode Island, where the 3 lines of American hog-peanuts were acquired, were 0.021, 0.098, and 0.039, respectively. The average probability of drought 0.53 was acquired by taking the arithmetic mean of the three values. In the simulations, this value is approximated at 0.5 while the probability of drought in an extremely dry year is approximated at 1.0.

Figure 2: Germination assays conducted on 600 aerial seeds in the Greenhouse

Line IDs				%Germinate
LG23	LG25	LG26	LG28	0.715

Right Half Tray

4Oct23	1Oct23	1Oct23		3Oct23		30Sep23	1Oct23	29Sep23	29Sep23
	30Sep23	30Sep23	29Sep23						1Oct23
30Sep23	2Oct23	1Oct23		1Oct23			30Sep23		29Sep23
3Oct23		1Oct23		29Sep23		29Sep23	3Oct23	30Sep23	29Sep23
30Sep23		5Oct23	5Oct23	29Sep23	29Sep23	1Oct23	29Sep23	3Oct23	29Sep23
	30Sep23	1Oct23	1Oct23	3Oct23	30Sep23	1Oct23	2Oct23		29Sep23
1Oct23		30Sep23	2Oct23			29Sep23		3Oct23	30Sep23
30Sep23	29Sep23	30Sep23			29Sep23	2Oct23	30Sep23		30Sep23
	29Sep23		1Oct23	29Sep23	29Sep23		10Oct23	29Sep23	30Sep23
	29Sep23	1Oct23	1Oct23	30Sep23	2Oct23		30Sep23	30Sep23	

Left Half Tray

			7Oct23	3Oct23	4Oct23		6Oct23	7Oct23	3Oct23
1Oct23	29Sep23		29Sep23		5Oct23		2Oct23	3Oct23	30Sep23
4Oct23		1Oct23	29Sep23	1Oct23			29Sep23	30Sep23	29Sep23
7Oct23	29Sep23			1Oct23	29Sep23	30Sep23		3Oct23	29Sep23
	29Sep23	29Sep23				2Oct23		30Sep23	29Sep23
29Sep23	29Sep23		1Oct23	29Sep23	29Sep23	29Sep23		2Oct23	1Oct23
29Sep23	29Sep23	2Oct23		2Oct23		29Sep23	29Sep23	1Oct23	1Oct23
29Sep23		30Sep23	29Sep23	29Sep23			29Sep23	29Sep23	30Sep23
29Sep23	29Sep23	30Sep23	29Sep23	29Sep23	29Sep23	29Sep23	29Sep23	29Sep23	2Oct23
4Oct23	29Sep23	2Oct23	29Sep23	29Sep23	30Sep23	29Sep23	30Sep23	30Sep23	

(a) *Aerial seeds from the LG line yielded a germination rate of 71.5%.*

Line IDs				%Germinate
LR12H.1B	LR12H.4B	LR12H.5A	LR12H.6A	0.71

Right Half Tray

25Sep23		30Sep23	23Sep23	25Sep23		23Sep23	24Sep23	24Sep23	26Sep23
29Sep23			23Sep23				23Sep23	27Sep23	25Sep23
	27Sep23		24Sep23	23Sep23	26Sep23	24Sep23		24Sep23	28Sep23
24Sep23	25Sep23	23Sep23	25Sep23		23Sep23	23Sep23	23Sep23	25Sep23	25Sep23
		24Sep23	4Oct23	30Sep23	24Sep23	23Sep23	30Sep23	29Sep23	27Sep23
			28Sep23	24Sep23	27Sep23	26Sep23		27Sep23	27Sep23
27Sep23	24Sep23	24Sep23	30Sep23	24Sep23	23Sep23	24Sep23	23Sep23	29Sep23	25Sep23
26Sep23	24Sep23		1Oct23	24Sep23	23Sep23	30Sep23	9Oct23	30Sep23	24Sep23
		24Sep23	23Sep23		30Sep23	24Sep23		27Sep23	23Sep23
			25Sep23	26Sep23	25Sep23	24Sep23	24Sep23	23Sep23	23Sep23

Left Half Tray

	24Sep23			26Sep23		24Sep23	24Sep23	24Sep23	26Sep23
	24Sep23		28Sep23	30Sep23			24Sep23	24Sep23	23Sep23
	24Sep23			25Sep23		1Oct23	30Sep23	23Sep23	
25Sep23	4Oct23	25Sep23	27Sep23	28Sep23				23Sep23	23Sep23
24Sep23	25Sep23	26Sep23				29Sep23	24Sep23	23Sep23	30Sep23
		27Sep23		25Sep23	24Sep23	29Sep23	28Sep23	23Sep23	23Sep23
		23Sep23	23Sep23		27Sep23		24Sep23	25Sep23	28Sep23
24Sep23		30Sep23	30Sep23	28Sep23	27Sep23	24Sep23	24Sep23	24Sep23	25Sep23
27Sep23	25Sep23	28Sep23		23Sep23		26Sep23	28Sep23	24Sep23	23Sep23
25Sep23					26Sep23	24Sep23	24Sep23	26Sep23	24Sep23

(b) *Aerial seeds from the LR line yielded a germination rate of 71.0%.*

Line IDs				%Germinate
KM1	KM2	KM3	KM4	0.975

Right Half Tray

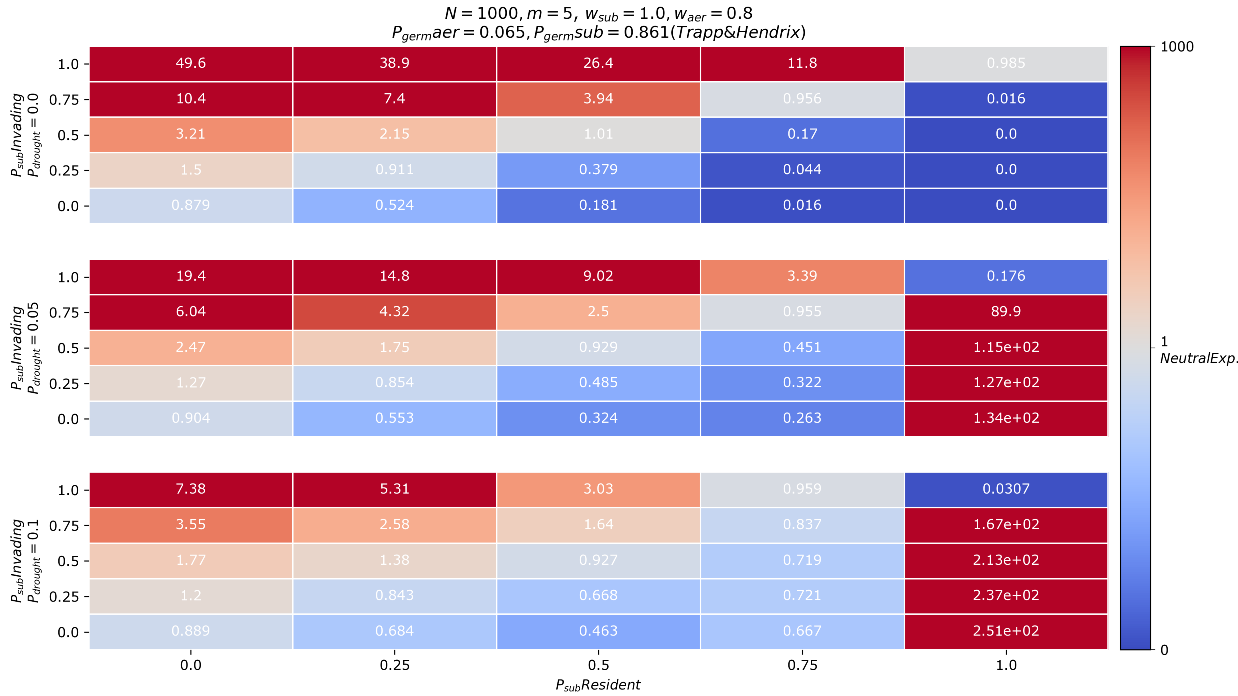
4Oct23	6Oct23	4Oct23	5Oct23	6Oct23	6Oct23	8Oct23	6Oct23	3Oct23	5Oct23
4Oct23	7Oct23	3Oct23	5Oct23	3Oct23	5Oct23	7Oct23	4Oct23	7Oct23	6Oct23
3Oct23	2Oct23	4Oct23	3Oct23	2Oct23	7Oct23	8Oct23	4Oct23	5Oct23	5Oct23
4Oct23	3Oct23	3Oct23	6Oct23	4Oct23	5Oct23	7Oct23	3Oct23	6Oct23	5Oct23
3Oct23	4Oct23	2Oct23	7Oct23	6Oct23	4Oct23		7Oct23	3Oct23	4Oct23
3Oct23	5Oct23	6Oct23	3Oct23	3Oct23	9Oct23	6Oct23	6Oct23	3Oct23	8Oct23
6Oct23	4Oct23	7Oct23	5Oct23	3Oct23	6Oct23	6Oct23	5Oct23	4Oct23	8Oct23
3Oct23	2Oct23	3Oct23	4Oct23	4Oct23	2Oct23	5Oct23	3Oct23	8Oct23	8Oct23
6Oct23	5Oct23	8Oct23	3Oct23	3Oct23	6Oct23	5Oct23	7Oct23	4Oct23	3Oct23
6Oct23	5Oct23	7Oct23	8Oct23	4Oct23	4Oct23	5Oct23	7Oct23	2Oct23	2Oct23

Left Half Tray

6Oct23	8Oct23	6Oct23	5Oct23	6Oct23	5Oct23	3Oct23	4Oct23	6Oct23	6Oct23
6Oct23	5Oct23	3Oct23	5Oct23	5Oct23	7Oct23	7Oct23	9Oct23	5Oct23	4Oct23
3Oct23	10Oct23	2Oct23	6Oct23	5Oct23	7Oct23	7Oct23	6Oct23	10Oct23	
4Oct23	2Oct23	2Oct23	5Oct23	4Oct23	7Oct23	7Oct23	5Oct23	3Oct23	8Oct23
5Oct23	2Oct23	7Oct23	6Oct23	5Oct23	5Oct23	5Oct23	8Oct23	6Oct23	3Oct23
5Oct23	5Oct23	5Oct23	7Oct23	4Oct23	6Oct23	5Oct23	6Oct23		
3Oct23	5Oct23	4Oct23	6Oct23	4Oct23	4Oct23	4Oct23	4Oct23	6Oct23	7Oct23
4Oct23	5Oct23	4Oct23	4Oct23	7Oct23	2Oct23	6Oct23	7Oct23		3Oct23
5Oct23	5Oct23	4Oct23	4Oct23	4Oct23	7Oct23	4Oct23	4Oct23	5Oct23	6Oct23
2Oct23	3Oct23	5Oct23	6Oct23	5Oct23	6Oct23	6Oct23	4Oct23	7Oct23	5Oct23

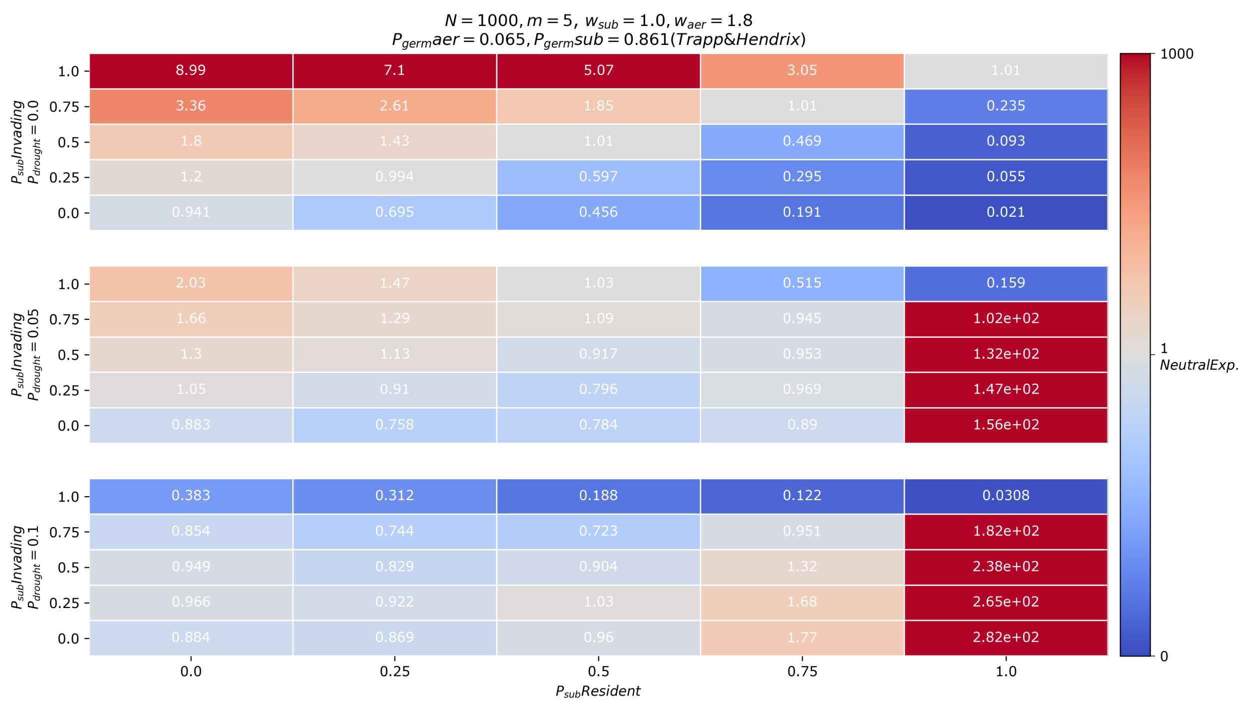
(c) *Aerial seeds from the KM line yielded a germination rate of 97.5%.*

Figure 3: Normalized probability of fixation for every combination of amphicarpic ratios with two different aerial fitness values, in a population of 1000 and a seed bank duration of 5 generations



$P_{drought}$	P_{sub}Invading \ P_{sub}Resident	0.0	0.25	0.5	0.75	1.0
0.0	1.0	49.6	38.9	26.4	11.8	0.985
0.0	0.75	10.4	7.4	3.94	0.956	0.016
0.0	0.5	3.21	2.15	1.01	0.17	0.0
0.0	0.25	1.5	0.911	0.379	0.044	0.0
0.0	0.0	0.879	0.524	0.181	0.016	0.0
0.05	1.0	19.4	14.8	9.02	3.39	0.176
0.05	0.75	6.04	4.32	2.5	0.955	89.9
0.05	0.5	2.47	1.75	0.929	0.451	1.15e+02
0.05	0.25	1.27	0.854	0.485	0.322	1.27e+02
0.05	0.0	0.904	0.553	0.324	0.263	1.34e+02
0.1	1.0	7.38	5.31	3.03	0.959	0.0307
0.1	0.75	3.55	2.58	1.64	0.837	1.67e+02
0.1	0.5	1.77	1.38	0.927	0.719	2.13e+02
0.1	0.25	1.2	0.843	0.668	0.721	2.37e+02
0.1	0.0	0.889	0.684	0.463	0.667	2.51e+02

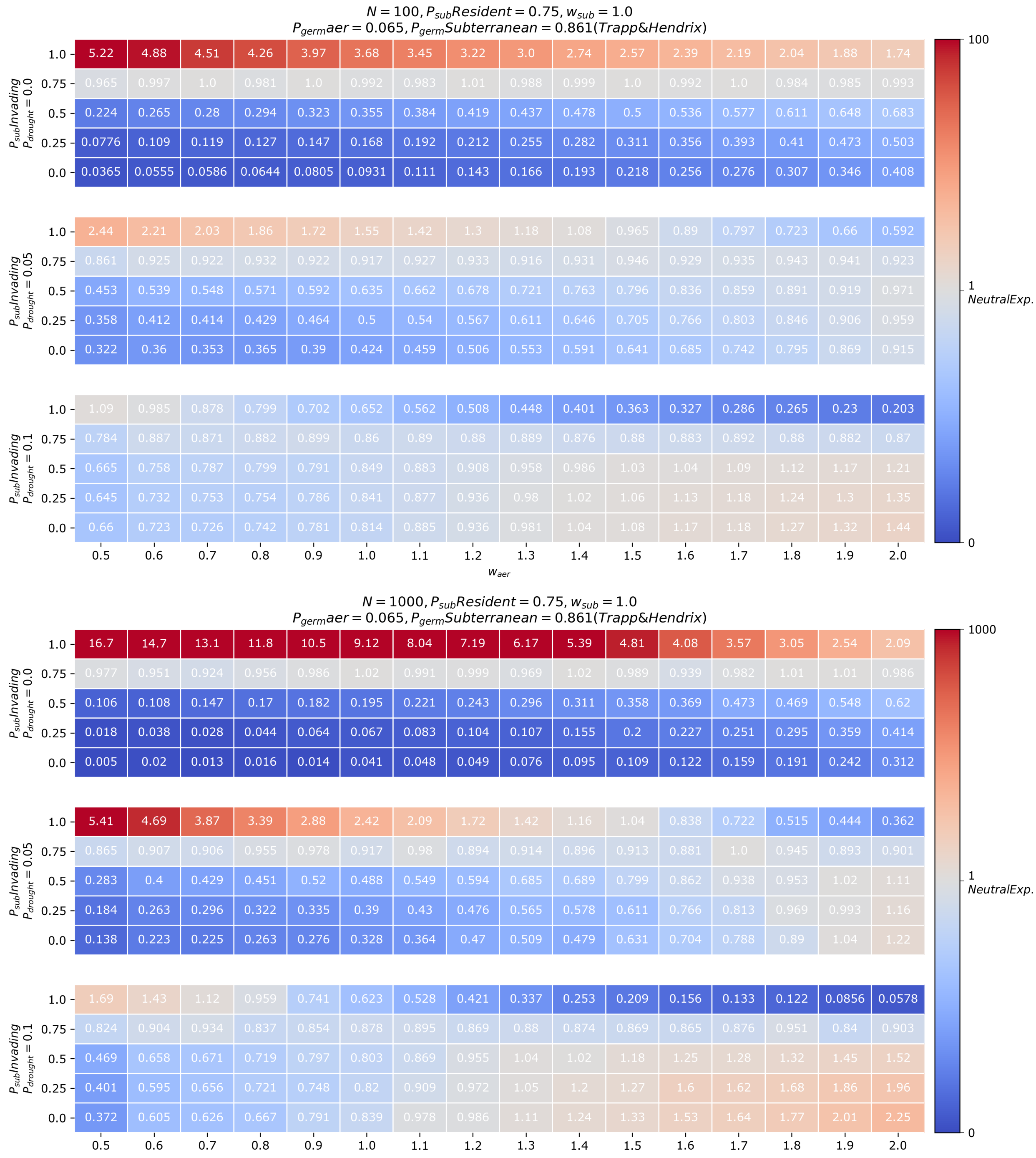
(a) *At $w_{aer} < 1.3$, monocarpy of subterranean seeds remains the optimal strategy.*



$P_{drought}$	P_{sub}Invading \ P_{sub}Resident	0.0	0.25	0.5	0.75	1.0
0.0	1.0	8.99	7.1	5.07	3.05	1.01
0.0	0.75	3.36	2.61	1.85	1.01	0.235
0.0	0.5	1.8	1.43	1.01	0.469	0.093
0.0	0.25	1.2	0.994	0.597	0.295	0.055
0.0	0.0	0.941	0.695	0.456	0.191	0.021
0.05	1.0	2.03	1.47	1.03	0.515	0.159
0.05	0.75	1.66	1.29	1.09	0.945	1.02e+02
0.05	0.5	1.3	1.13	0.917	0.953	1.32e+02
0.05	0.25	1.05	0.91	0.796	0.969	1.47e+02
0.05	0.0	0.883	0.758	0.784	0.89	1.56e+02
0.1	1.0	0.383	0.312	0.188	0.122	0.0308
0.1	0.75	0.854	0.744	0.723	0.951	1.82e+02
0.1	0.5	0.949	0.829	0.904	1.32	2.38e+02
0.1	0.25	0.966	0.922	1.03	1.68	2.65e+02
0.1	0.0	0.884	0.869	0.96	1.77	2.82e+02

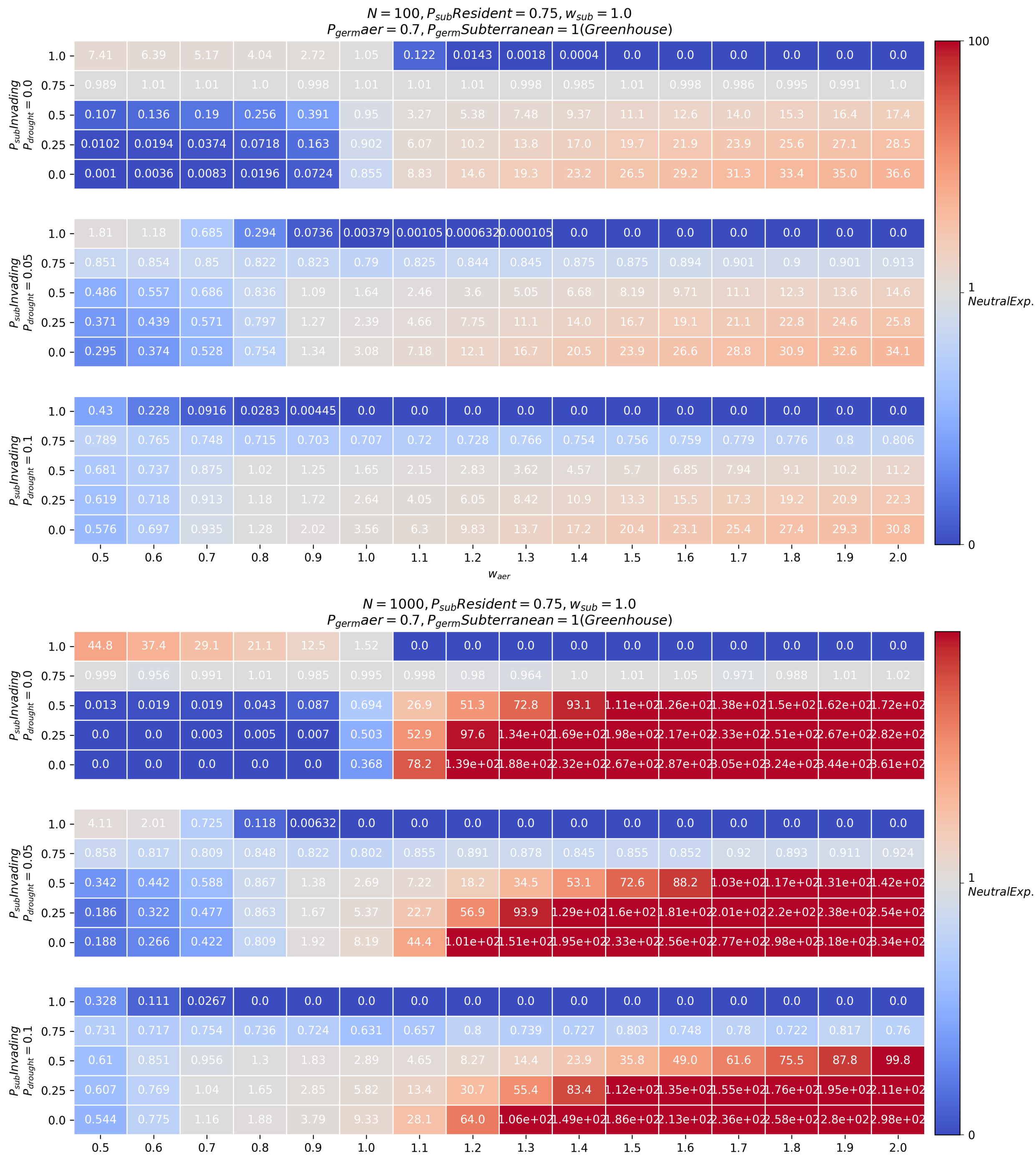
(b) *At $w_{aer} > 1.3$, amphicarpy starts to appear beneficial at low (realistic) amphicarpic ratios under severe drought.*

Figure 4: Normalized probability of fixation for every combination of amphicarpic ratios within a range of aerial fitness values with natural germination rates, with a seed bank duration of 5 generations



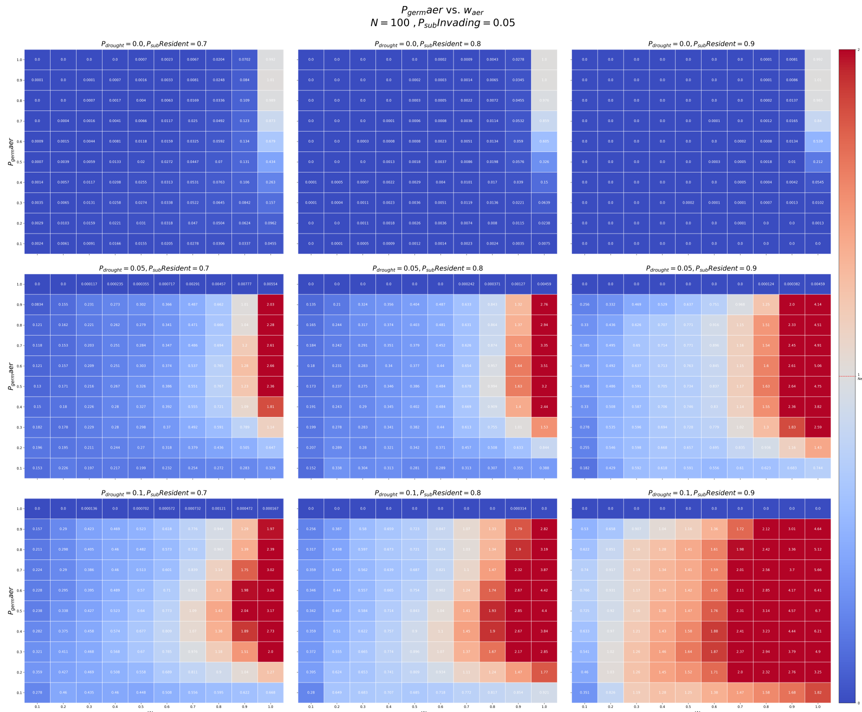
In populations of varying sizes, amphicarpy remains beneficial at low (realistic) amphicarpic ratios after some w_{aer}. This w_{aer} value slightly varies across population sizes.

Figure 5: Normalized probability of fixation for every combination of amphicarpic ratios within a range of aerial fitness values with Greenhouse germination rates, with a seed bank duration of 5 generations

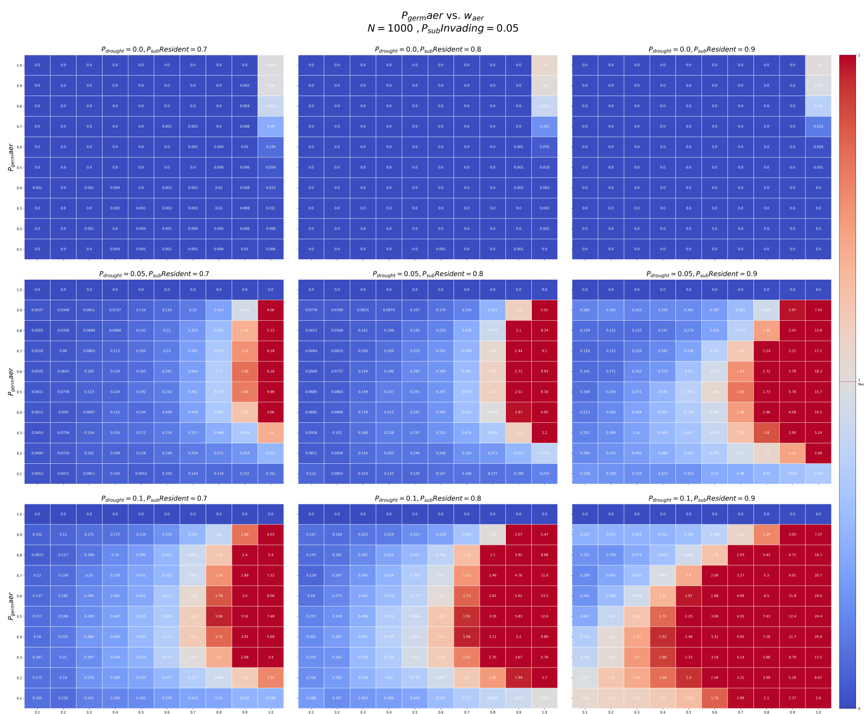


In populations of varying sizes, amphicarpy remains beneficial at low (realistic) amphicarpic ratios after some w_{aer}. This w_{aer} value slightly varies across population sizes.

Figure 6: Normalized probability of fixation for a low amphicarpic ratio = 0.05, surveyed across a range of aerial fitness values and a range of germination rates, with a seed bank duration of 5 generations

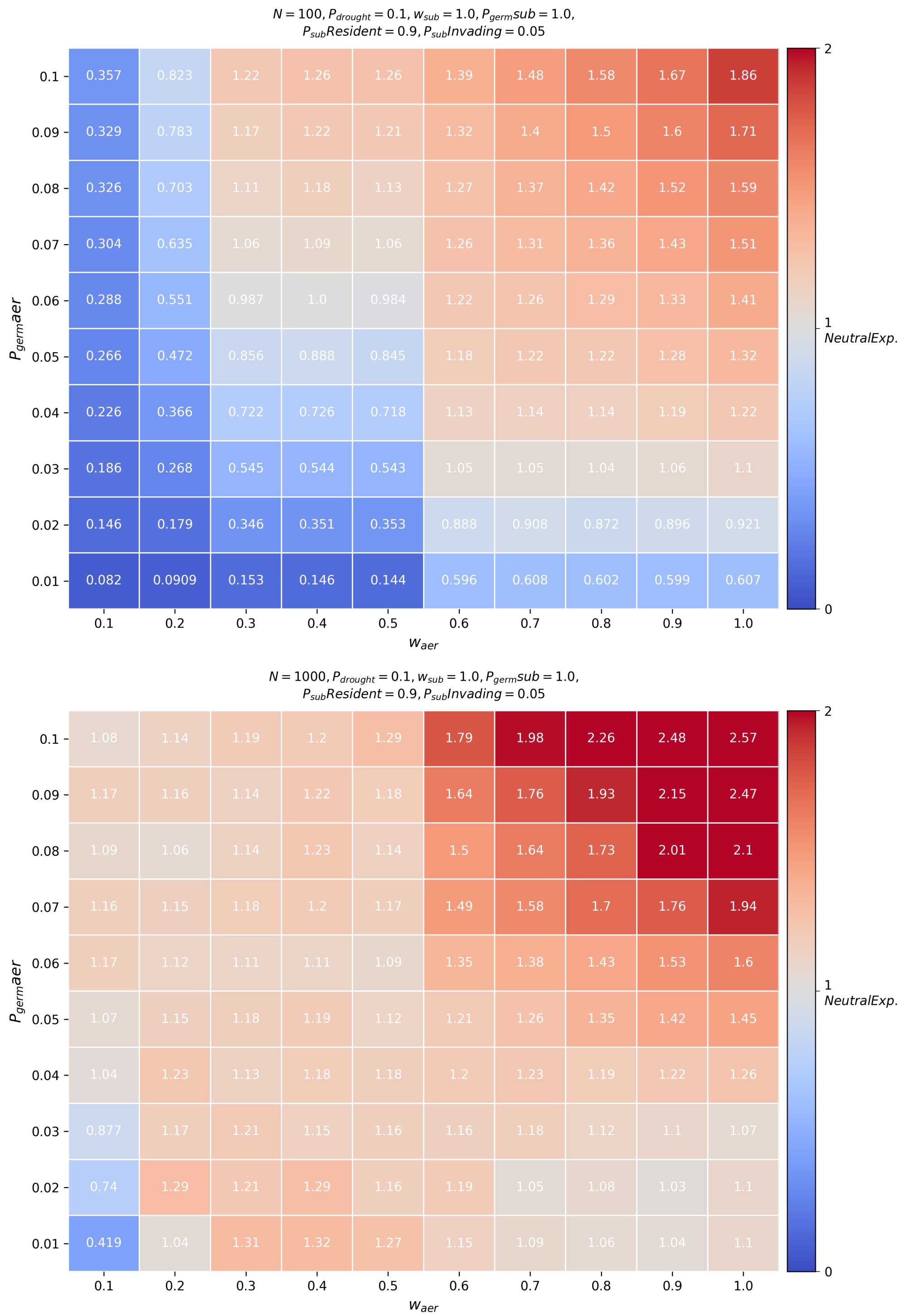


(a) *In a population of 100, the region of parameter space where amphicarpy is beneficial expands with increasing severity of drought as well as increasing proportion of subterranean seeds produced by the Resident population.*



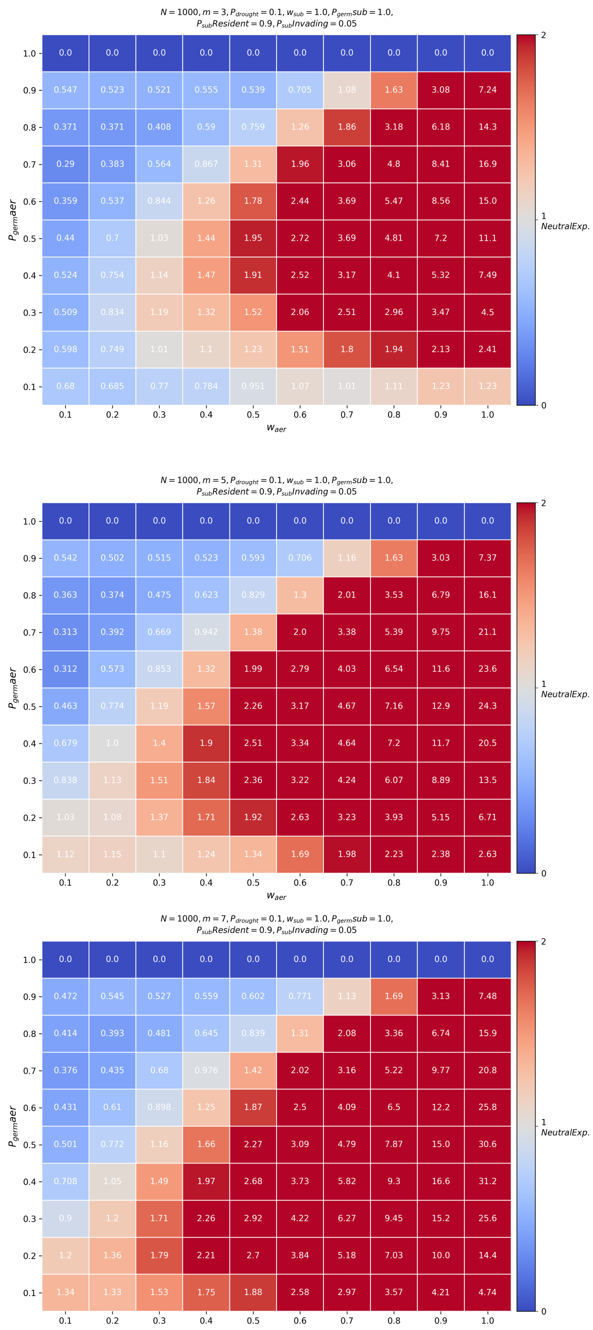
(b) *In a population of 1000, the same trend is observed as in (a) but the threshold germination rate and threshold fitness have both decreased.*

Figure 7: Normalized probability of fixation for a low amphicarpic ratio = 0.05, surveyed across a range of aerial fitness values and a fine range of germination rates in [0, 0.1], in a population of 1000 with a seed bank duration of 5 generations

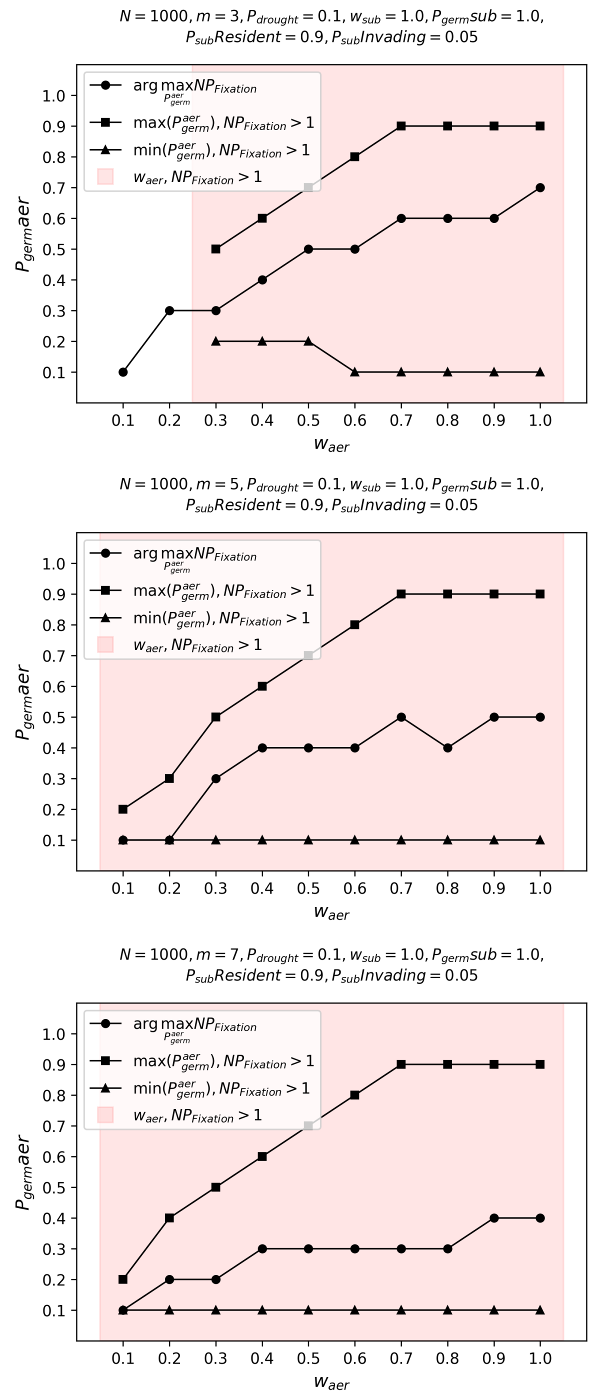


Under severe drought, germination rate finds its threshold at around 0.1. Although small, the regions where normalized probability of fixation ¡ 1 still exist at the left bottom corner of the parameter space, suggesting that the non-linear relationship between $NP_{Fixation}$ and the combined effect of w_{aer} and $P_{germ^{aer}}$ still holds. An increase in population size expands the beneficial region in the parameter space.

Figure 8: Normalized probability of fixation for a low amphicarpic ratio = 0.05, surveyed across a range of aerial fitness values and germination rates, in a population of 1000 under severe drought with variable seed bank durations (m)



(a) *Beneficial region in the parameter space at $m = 5$ (aerial seeds allowed for 3 generations in the seed bank) has expanded compared to that at $m = 3$. Although the beneficial region at $m = 7$ is very similar to that at $m = 5$, normalzied probability of fixation has generally increased.*



(b) *As m increases, the germinate rate that maximizes normalized probability of fixation decreases. The germinate rates that constitute the upper and lower bound of this beneficial region expands in range.*