

# The emergence and evolutionary fate of diversity under soft and hard selection

Patrick Chen

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## **Abstract**

It has long been appreciated that genetic diversity can be stably maintained in spatially structured environments, depending on the method by which subdivided populations are regulated. Theory suggests diversity in spatially heterogeneous environments can be stably maintained when populations are regulated at the level of each individual subpopulation (soft selection) rather than at the level of the total mixed population (hard selection). Although mathematically established decades ago, direct empirical tests of these predictions remain scarce, and their robustness to prolonged selection on evolutionary timescales remain unclear. Here, we track the emergence and fate of diversity in replicate laboratory populations of the opportunistic human pathogen, *Pseudomonas aeruginosa*, evolving in spatially structured environments composed of patches with or without antibiotic selection. We manipulate the scale of population regulation by transferring either a fixed number (soft selection) or volume (hard selection) of cells. We find that, while soft and hard selection can lead to prolonged coexistence between resistant and sensitive strains, diversity is eventually lost by the evolution of broadly adapted resistant strains that pay lower costs of resistance. Notably, coexistence on average persists longer under soft selection than hard selection, consistent with the idea that soft selection is more effective at maintaining diversity than hard selection. Our results suggest that, in line with theory, the manner of population regulation can impact the maintenance of diversity and that the ecological conditions supporting diversity in the short term can be readily undermined by evolutionary processes in the long term.

## Résumé

La diversité génétique peut être maintenue de façon stable dans le contexte d'un environnement ayant une structure spatiale suivant la manière selon laquelle les sous-populations sont régulées. Les travaux théoriques indiquent que, dans un environnement ayant une hétérogénéité spatiale, cette diversité peut être maintenue lorsque la régulation a lieu au niveau de chaque sous-population (la sélection douce), contrairement à lorsqu'elle a lieu au niveau de la population totale (la sélection dure). Quoiqu'elles soient éprouvées mathématiquement depuis des décennies, ces prédictions ont rarement été testées de façon empirique ; de plus, leur robustesse face à une sélection prolongée, de l'ordre de périodes évolutives, demeure inconnue. Ici, nous suivons l'émergence et le sort de la diversité chez des populations répétées de *Pseudomonas aeruginosa*, un pathogène humain opportuniste, lorsqu'elles évoluent dans des milieux ayant une structure spatiale composée de zones avec et sans sélection aux antibiotiques. Nous manipulons l'échelle de la régulation des population en modulant la quantité de cellules transférées : soit un nombre fixe de cellules (la sélection douce), soit un nombre flou, c'est-à-dire par volume (la sélection dure). Bien que la coexistence prolongée de lignées résistantes et sensibles persiste dans les deux cas, la diversité disparaît finalement dû à l'émergence de lignées globalement résistantes n'ayant que peu de coûts évolutifs associés à cette résistance. La coexistence dure notamment plus longtemps dans le protocole de sélection douce, en accord avec l'idée que la sélection douce maintient la diversité plus efficacement que la sélection dure. Nos résultats indiquent que, conforme aux théories, la façon selon laquelle une population est régulée a un impact sur le maintien de la diversité, et que les conditions écologiques soutenant la diversité à court terme peuvent être aisément sapées par des processus évolutifs à long terme.

## Table of Contents

Abstract	ii
Résumé	iii
Table of Contents	iv
List of Tables	v
List of Figures	vi
Acknowledgements	vii
<b>General Introduction</b>	<b>1</b>
<b>Chapter 1: The emergence and evolutionary fate of diversity under soft and hard selection</b>	
<i>Introduction</i>	4
<i>Methods</i>	6
<i>Statistical Analysis</i>	9
<i>Results</i>	10
<i>Discussion</i>	12
<b>Conclusion</b>	<b>14</b>
Tables	16
Figures	20
References	23

## **List of Tables**

### **Table 1.**

Generalized linear analysis of covariance for comparing the proportion of resistant individuals between soft and hard selection through time.

### **Table 2.**

Mixed linear analysis of covariance for comparing the relative fitness of resistant types between day 10 and day 40

### **Table 3.**

Mixed linear analysis of covariance for comparing growth rate-resistance trade-offs between soft and hard selection.

### **Table 4.**

Mixed linear analysis of covariance for comparing trade-offs associated with resistance between soft and hard selection.

## **List of Figures**

### **Figure 1.**

The proportion of resistant individuals within soft selected and hard selected populations.

### **Figure 2.**

Negative frequency dependent selection within hard selected or soft selected populations on day 10 and day 40.

### **Figure 3.**

The trade-off between growth rate in permissive LB, and resistance of eight isolates taken from soft selected or hard selected lines on days 10, 20, 30, and 40 of the experiment.

### **Figure 4.**

The relative fitness of resistant isolates and sensitive isolates from hard selected populations and soft selected populations on day 10 and day 40

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## General Introduction

Genetic variation is essential for the process of adaptation and provides the raw materials upon which evolution works. While the amount of variation within a population is predicted to decrease through time, as selection should favour only the few genotypes that with the greatest fitness, this prediction is seemingly contradicted by the abundance of variation that persists within natural populations. This dilemma highlights the need for a better understanding of the forces that govern the maintenance and the ultimate fate of variation within populations. As such, this remains one of the most crucial topics in the field of evolutionary biology.

The large amount of variation observed within natural populations has long been proposed to be the result of several processes, such as mutation-selection balance, neutral variation governed by drift, and balancing selection. In the case of mutation-selection balance, the variation observed within a population has been suggested to be a result of the persistent introduction of deleterious mutations into the population and their subsequent purge by selection (Barton 1986). However, not all mutations confer beneficial or negative fitness effects, and neutral mutations, whose frequency and fate are governed by genetic drift, could also contribute to the variation observed in populations (Kimura 1979). While these stochastic processes contribute to diversity through the introduction and persistence of genetic variation within populations, balancing selection, underlain by environmental heterogeneity, has long been thought to be an important driver of the maintenance of standing genetic variation (Hedrick *et al.* 1976; Hedrick 1986). Under balancing selection, genetic variation within a population is maintained when genotypes are adapted to different sites, or patches, available within a variable environment. Although the effects of environmental heterogeneity on variation within populations has been extensively studied (Rainey & Travisano 1998; Jasmin & Kassen 2007; Huang *et al.* 2016; Leale & Kassen 2018), the underlying mechanism that maintains this variation remains largely untested. My research focuses on two classical models of balancing selection, the Levene model (Levene 1953) and the Dempster model (Dempster 1955), which make predictions about the fate of diversity within populations in a spatially heterogeneous environment.

These models describe a population that is subdivided within a coarse-grained, heterogeneous environment, where individuals of the population reside and experience selection from a single

patch throughout their lifetimes (Levins 1968). Each generation, a migration event occurs where the offspring from the whole population come together, mix, and are randomly dispersed among the patches. The two models differ in the manner in which density regulation is enforced upon the population. In the Levene model (Levene 1953), density regulation is applied locally at each individual patch, such that the contribution of each patch to the next generation is fixed, regardless of relative patch productivity and their genotypes. As a result, the fitness of individuals is relative to other genotypes within each patch. This has come to be known as soft selection (Christiansen 1975; Wallace 1975). In contrast, in the Dempster model (Dempster 1955), density regulation occurs at the scale of the whole population following global pooling and before dispersal. Consequently, under hard selection, individuals that are better adapted in a highly productive patch contribute disproportionately to the next generation (Christiansen 1975; Wallace 1975).

This subtle difference in the manner of population regulation has been predicted to have a profound effect on the fate of diversity. Hard selection cannot support diversity, as the fittest individuals from the most productive patch will eventually invade other patches and swamp out the other types. In comparison, as the contribution of each patch is fixed under soft selection, diversity has been predicted to be stably maintained under soft selection (Karlin & Campbell 1981; Wade 1985), through negative frequency dependent selection (Strobeck 1974; Clarke 1979). However, density regulation alone is not sufficient to maintain diversity; diversity can be readily undermined by the introduction of a broadly adapted generalist which will come to invade and dominate the landscape. Therefore, in addition to soft selection, strong fitness trade-offs across environments (Levins 1962; Reboud & Bell 1997; Kassen 2002), or low levels of migration loads are necessary to prevent the loss of diversity (Smith 1966; Débarre & Gandon 2011).

Although the conditions necessary for the stable maintenance of diversity under soft selection are stringent (Prout 1968; Christiansen 1974; Smith & Hoekstra 1980), the model of soft selection provides the framework upon which several theoretical studies have further expanded to include habitat choice, intermediate levels of density regulation, and polygenic traits (Garcia-Dorado 1986; Spichtig & Kawecki 2004; Débarre & Gandon 2011). However, empirical tests of the hard and soft selective models are few, and at the time of this writing, only one recent study (Gallet *et*

*al.* 2018) has successfully provided empirical support for the models on ecological timescales, in the absence of adaptation. While the ecological outcome of diversity has been explored extensively in the literature, the evolutionary outcome of diversity under soft selection has received much less attention( Kisdi & Geritz 1999; Meeûs & Goudet 2000; Kisdi 2001), and to my knowledge, remains untested.

Drawing on the designs of two studies that had previously empirically tested the Levene and Dempster models on ecological timescales (Bell 1997; Gallet *et al.* 2018), my thesis aims to study the effects of soft and hard selection on the emergence, maintenance and evolutionary fate of diversity within populations subdivided among spatially heterogeneous environments, and the underlying mechanisms that drive the maintenance of diversity under soft selection. The key findings of my research are detailed in Chapter 1, an article coauthored with my supervisor Dr. Rees Kassen that will be submitted to *Ecology Letters*.

## **Chapter 1: The emergence and evolutionary fate of diversity under soft and hard selection**

### **Introduction**

Genetic variation is essential for the process of adaptation and provides the raw materials upon which evolution works. As such, identifying and understanding the forces that govern the maintenance and ultimate fate of variation within populations remains a crucial topic in the field of evolutionary biology. A considerable amount of variation observed in nature can be attributed to environmental heterogeneity, and in particular, spatial heterogeneity (Rainey & Travisano 1998; Jasmin & Kassen 2007; Huang *et al.* 2016; Leale & Kassen 2018). Although several forces, such as migration (Smith 1966; Débarre & Gandon 2011) and the strength of divergent selection (Levins 1962; Reboud & Bell 1997; Kassen 2002) govern whether diversity is maintained or lost through time, special attention has been paid to the manner in which density regulation is applied to subdivided populations. There exist two classical models, the models of soft and hard selection (Levene 1953; Dempster 1955; Wallace 1975), that address the effect of density regulation on the fate of diversity.

These models describe a population that is subdivided within a coarse-grained, heterogeneous environment, where individuals of the population experience selection from a single habitat, or patch, throughout their lifetime (Levins 1968). Every generation, the population experiences a panmictic migration event, where the offspring from the whole population are mixed and randomly dispersed among the patches. In the model of soft selection (Levene 1953; Christiansen 1975; Wallace 1975), density regulation occurs at the level of each independent patch, prior to population mixing. In contrast, under hard selection (Dempster 1955; Christiansen 1975; Wallace 1975), density regulation occurs at the scale of the whole population following global pooling.

The subtle difference in the scale at which population regulation is imposed can have profound effects on the opportunity for representation in the following generation, and therefore the fate of diversity. Under hard selection, individuals that are better adapted in a highly productive patch contribute disproportionately to the next generation, and diversity is not expected to be maintained. However, under soft selection, the contribution of each patch to the next generation is fixed, and therefore the fitness of an individual within each patch is relative to the quality of others within its patch (Karlin & Campbell 1981; Wade 1985). It is under soft selection that

diversity is predicted to be maintained, through negative frequency dependent selection (Strobeck 1974). Although the model of soft selection provides the framework for much of the current theoretical literature regarding the maintenance of diversity (Felsenstein 1976; Garcia-Dorado 1986; Spichtig & Kawecki 2004; Débarre & Gandon 2011), it was only recently that a direct empirical study provided support for the predictions of the model (Gallet *et al.* 2018). The study by Gallet *et al.*, demonstrates that diversity is maintained in populations undergoing soft selection maintains diversity in a frequency dependent fashion, whereas diversity is lost in populations undergoing hard selection. However, this test was done on an ecological timescale, in the absence of adaptation, and whether diversity is maintained under soft selection through negative frequency dependent selection was not explored in detail. The question of whether the models of soft and hard selection can be applied on evolutionary timescales has received little attention (Kisdi & Geritz 1999; Meeûs & Goudet 2000; Kisdi 2001), and to our current knowledge, has remained untested.

Here, we examine the predictions made by the soft and hard selection models on evolutionary timescales. To do this, we propagated 24 independent, isogenic, replicate populations of the gram-negative bacteria *Pseudomonas aeruginosa*. Each experimental population was subdivided in a spatially heterogeneous landscape, such that half experiences an antibiotic selective pressure, and the other does not. Population regulation and mixing was applied via serial passages to emulate either hard selection (by transferring equal aliquots of each subpopulation) or soft selection (by transferring an equal number of cells from each subpopulation). To examine the evolutionary effects of soft or hard selection, we allowed the variation within the population to arise through mutations and evolve. We made comparisons in the response to antibiotic selection by tracking the evolution of resistance over a course of approximately 264 generations (40 days).

Taken together, our results provide the first direct experimental test of the soft and hard selection models on both ecological and evolutionary timescales. Our findings are in line with theory, in that soft selection can stably maintain diversity through negative frequency dependent selection, while hard selection does not. However, we also found evidence for the evolution of broadly adapted generalists under both selection regimes, suggesting that although soft selection maintains diversity for a longer period of time than hard selection, diversity will not be protected indefinitely.

## **Methods**

### **Study system**

Isolates of *P. aeruginosa* strains, either PA14 or an isogenic PA14 with an insertion of the neutral *lacZ* gene, were used to found a total of 24 replicate populations. Colonies with the *lacZ* gene appear blue when cultured on agar plates supplemented with 40 mg L<sup>-1</sup> of 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal), and are visually distinct from the wild type white colouration. Populations of both strains were equally distributed into either a soft or hard selective regime. Both ancestral clones were archived in a 16% glycerol solution at -80°C.

Populations were subdivided into two subpopulations such that half of the population was grown in lysogeny broth (LB: bacto-tryptone 10g/L, yeast extract 5g/L, NaCl 10g/L) and half was grown in LB supplemented with 0.3 µg/ml of the fluoroquinolone antibiotic, ciprofloxacin. This concentration of ciprofloxacin was previously determined to reduce maximal growth of a sensitive PA14 ancestor to 20% of full growth in LB over a 24h period. Populations were routinely screened every 3-4 days (20-26 generations) for cross contamination before being archived in a 16% glycerol solution at -80°C.

### **Selection treatment**

Soft selection was imposed by regulating the cell densities of subpopulations prior to population mixing and dispersion. This was done by comparing the optical densities of each subpopulation using a spectrophotometer (Elx-800: BioTek Instruments Inc., Winooski, VT) and diluting with plain LB until the optical densities were approximately equal. 500ul samples were taken from each subpopulation to generate a mixed population. Hard selection was imposed by taking 500ul samples from each subpopulation to generate a mixed population. All mixed populations from both selection regimes were diluted to a common density prior to dispersion. The selection experiment was propagated for 40 days (264 generations).

### **Proportion of resistance**

We tracked the proportion of resistant individuals within each evolved population on days 1, 3, 5, 10, 15, 20, 30 and 40 in order to contrast the rate at which resistant genotypes spread within populations between selective treatments. We estimated the proportion of resistant individuals by isolating 50 random individual colonies from each mixed population and testing each for

resistance to ciprofloxacin at a concentration of 2 $\mu$ g/ml on LB agar plates. The concentration of ciprofloxacin used here was experimentally determined to inhibit growth of the ancestral strain on LB agar plates. The proportion of resistance for each population was expressed as the number of resistant colonies out of the 50 selected.

### **Trade-offs**

Diversity is easily undermined by the evolution of a generalist that can easily invade and dominate the landscape as a result of low costs for resistance traits. To investigate the emergence of these generalists, we examined trade-offs between growth rate and resistance. We measured the minimum inhibitory concentration (MIC) and the maximal growth rate in LB of eight chosen isolates (4 sensitive, 4 resistant) from each replicate population on days 10, 20 30 and 40. Each isolate was grown for 24h in LB supplemented with the following concentrations of ciprofloxacin: 0, 0.2, 0.4, 0.8, 1.6, 3, 6, 12, 25, 30, 40 and 50 $\mu$ g/ml. MIC was determined by visual inspection, and confirmed by comparing the optical density of the wells with LB media. Growth rate was determined separately by inoculating 5ul of dense, overnight culture of the same eight isolates from each population into 195ul of LB, and progressively monitoring the optical density of each culture at intervals of 90 minutes over a span of 24 hours. Estimates for maximal growth rates were determined using Gen5 software (BioTek Instruments Inc., Winooski, VT), and were expressed relative to the growth rate of an ancestor grown on the same 96-well plate.

### **Negative frequency dependent selection**

In order to test whether negative frequency dependent selection is the mechanism by which soft selection maintains diversity, we used a reciprocal competitive invasion experiment using isolates taken from days 10 and day 40. From each population, one resistant and one sensitive isolate were selected at random and competed by mixing overnight culture samples at ratios of 1:9, 1:1 and 9:1 by volume. Competitions lasted for two transfer cycles (48hrs) under the same treatment protocol from which the isolates were taken from. We estimated the frequency of resistant colonies by plating at least 50 random individual colonies prior to, and after, the competition on LB agar plates supplemented with ciprofloxacin at a concentration of 2 $\mu$ g/ml. The relative fitness of resistant isolates was estimated using the equation:

$$\omega = \left( \frac{R_{final}}{R_{initial}} \right)^{\frac{1}{doublings}}$$

where  $R_{initial}$  and  $R_{final}$  represent the ratios of the frequency of resistant types to the frequency of sensitive types, before and after the competition, respectively. Doubling refers to the number of generations between the initial and final measurements (approximately 13 generations; ~6.6 generations per 24h cycle).

### **Costs of resistance**

In order to have a broader measurement for the costs of resistance, we randomly selected a sensitive and resistant isolate from each line on day 10 and day 40, and contrasted the relative fitness when competed against a common ancestor. Competitions were done by mixing dense culture from an isolate and a reciprocally marked ancestor at a 1 : 1 ratio by volume and inoculating into plain LB. Competitions lasted for 24h. We tracked the change in relative fitness from initial (0hrs) to final (24hrs) by plating initial and the final culture competitions onto LB agar plates and visually scoring for the focal strain. The relative fitness of focal isolates was estimated using the equation:

$$\omega = \left( \frac{F_{final}}{F_{initial}} \right)^{\frac{1}{doublings}}$$

where  $F_{initial}$  and  $F_{final}$  represent the ratios of the frequency of the evolved population to the frequency of the marked ancestor, before and after the competition, respectively. Doubling refers to the number of generations between the initial and final measurements (approximately 6.6 generations).

## Statistical Analysis

All statistical analyses were conducted using R (version 3.3.1) with package lme4 (Bates *et al.* 2015). To compare the effect of soft or hard selection on the spread of resistance throughout the experiment, we ran a generalized linear model with a binomial (logit) link.

*glm: Proportion of resistance = Treatment \* Day + 0*. A fixed intercept at the origin was used because the initial progenitors used to propagate the populations in this experiment were fully susceptible to the level of antibiotic used.

To contrast the trade-offs between maximum growth rate and the strength of antibiotic resistance (log(MIC)) at four timepoints, we used a linear mixed model, with a random effect of population to account for repeated measures. *lmer: Growth rate = LogMIC \* Treatment \* Day + (1|Population)*. Evidence indicating the presence of a trade-off between growth rate and log MIC is given by a negative correlation.

Linear mixed models were also used to model the strength of negative frequency dependent selection at two timepoints. Relative fitness ( $\omega$ ) was modeled as a function of the initial frequency of resistance, a random intercept of individual population to account for repeated measures. *lmer:  $\omega = Frequency_{initial} * Day + (1|Population)$* . Evidence for negative frequency dependence would be given if there is a negative correlation between relative fitness of resistant isolates and the initial frequency of resistance.

Linear mixed models were again used to contrast the relative fitness of resistant and sensitive individuals across treatments at two timepoints; a random effect of population was also included. *lmer:  $\omega = Treatment * Resistant Or Sensitive * Day + (1|Population)$* . Evidence of a trade-off is given by the difference in relative fitness between sensitive and resistant isolates. Contrasts in the difference in relative fitness between sensitive and resistant isolates across timepoints were used to examine how trade-offs evolved through time.

## Results

### Rate of adaptation between soft and hard selection

We found the rate at which resistance propagated through the populations was significantly higher under hard selection than soft selection ( $P < 0.001$ ; **Table 1**; **Fig. 1**). Resistance spread through the populations non-linearly. By the end of the experiment, populations under hard selection had, on average, a greater proportion of resistance than soft selection ( $P < 0.00001$ ; **Table 1**).

### Maintenance of diversity through negative frequency dependent selection

A positive signal for the presence of negative frequency dependent selection would be given by a negative correlation between the relative fitness of resistant individuals and the initial frequency of resistance. Our results show that populations under soft selection demonstrate a signal for negative frequency dependent selection on both day 10 (**Table 2**; **Fig. 2**) and day 40 (**Table 2**; **Fig. 2**). Furthermore, our results show that populations under hard selection also demonstrate a signal for negative frequency dependent selection at day 10 (**Table 2**; **Fig. 2**), however this signal is no longer present by day 40 (**Table 2**; **Fig. 2**).

### Trade-offs between growth rate and MIC

Populations under soft selection demonstrated a trade-off between growth rate and logMIC for a greater period of time than those under hard selection (**Table 3**; **Fig. 3**). Our results show that the trade-off, as shown by the negative correlation between growth rate and MIC, was present within both selective regimes on day 10 and 20. However, this trade-off was no longer present under hard selection by day 30 (**Table 3**; **Fig. 3**), followed by soft selection on day 40 (**Table 3**; **Fig. 3**). This trend, under both soft and hard selection, appears to be driven by the evolution of lower cost resistance rather than a decrease in growth rate of the sensitive isolates.

### Costs of resistance

Using a single component of fitness to measure trade-offs provides a limited scope for examining the costs of resistance. To provide a more comprehensive test for costs of resistance, we contrasted the relative fitness of sensitive or resistant isolates when competed against a common ancestor (**Fig. 4**, **Table 4**). We found that there were costs for resistance under both selection regimes, and that this cost was present throughout the experiment (**Table 4**). However, we found

that the cost was lower at day 40 under hard selection than under soft selection. Furthermore, we also found that the resistant isolates taken from both soft and hard selective lines had evolved lower-cost resistance by day 40.

## Discussion

Theory predicts that under soft selection, where the contribution of each patch to the next generation is constant, diversity will be protected through negative frequency dependent selection. In contrast, under hard selection, the contribution to the next generation is determined by inherent patch productivity, so diversity is not expected to be maintained (Christiansen 1974; Karlin & Campbell 1981). In this present study, we tested whether diversity is maintained through negative frequency dependent selection, and whether the predictions made by the model on the fate of diversity can be extended to include both ecological and evolutionary timescales.

We found that the rate at which resistant genotypes propagate within populations is faster under hard selection than soft selection, and that the average proportion of resistance tended to be higher under hard selection. We take this as support for the idea that soft selection is more effective than hard selection at maintaining diversity, by protecting sensitive individuals from being outcompeted by resistant individuals. However, we note that there is a strong similarity between the trends observed between soft and hard selection. While it is largely understood that the conditions for soft selection to maintain diversity are stringent (Prout 1968; Christiansen 1974; Smith & Hoekstra 1980), it is more realistic to consider deviations caused by drift and mutation to cause selection to be less soft or hard than originally predicted by the models. Regardless, our results still lend support for the idea that soft selection better protects diversity in the long run.

A crucial condition for the stable maintenance of diversity under soft selection is a negative genetic correlation in fitness across environments. The importance of these trade-offs is highlighted by a previous study (Bell 1997), where the absence of strong trade-offs led to inconclusive results when contrasting the effects of hard and soft selection. While we found evidence for a trade-off between growth rate and resistance under soft selection, this signal did not persist for the entirety of the experiment. We hypothesized that the costs of resistance were still present in other fitness traits, and that using only a single component of fitness provided a very narrow scope for examining these costs. Reassuringly, by using a broader method of measuring fitness, through the competitive fitness assays against a common ancestor, we found that trade-offs in fitness were still present late throughout the experiment under soft selection.

Theory predicts that the stable maintenance of diversity under soft selection would largely be driven by negative frequency dependent selection. In line with theory, and a recent study (Gallet

*et al.* 2018), we observed a signal for negative frequency dependent selection under soft selection. Furthermore, this signal persisted for the duration of the experiment, suggesting that soft selection is capable of maintaining diversity for long periods of evolutionary time. Interestingly, we had also detected a signal for negative frequency dependent selection under hard selection, although this was transient and was not present at the end of the experiment. This was an unexpected result, as hard selection is expected to never maintain diversity (Christiansen 1974; Meeûs *et al.* 1993). This outcome could have been the result of subpopulations under hard selection having reached similar densities at the time when the subpopulations were sampled, leading to selection similar to soft selection being applied. Despite this, there is clear evidence that hard selection is incapable of maintaining diversity for a period of evolutionary time comparable to soft selection.

We are the first to empirically demonstrate that populations undergoing soft selection are capable of protecting diversity over a substantial amount of evolutionary time. Previous theoretical studies have addressed this question, and made predictions for the conditions under which soft selection may protect polymorphisms over the long-term (Kisdi & Geritz 1999; Meeûs & Goudet 2000; Kisdi 2001). We have also demonstrated, through an evolution experiment, that soft selection generates conditions that maintain diversity through negative frequency dependent selection, as predicted by theory (Strobeck 1974), and that it is necessarily underlain by costs of adaptation across environments. However, there is evidence for the evolution of low-cost resistant genotypes under both soft and hard selection. This leads us to suggest that while soft selection may preserve diversity for a longer period of evolutionary time than hard selection, eventually genotypes that low costs for resistance will come to dominate the landscape under both conditions.

Our study highlights the importance of evolutionary processes in predicting the fate of diversity, and offers greater insight on more applicable topics. One example could be the management of antimicrobial resistance within structured environments (Vale 2013; Leale & Kassen 2018). Although this study does not address the relationship between host and pathogen, it offers insight on the factors that maintain genetic diversity within patchy environments, which can aid in management of the evolution of antimicrobial resistance.

## Conclusion

Understanding factors that govern the emergence, maintenance and ultimate fate of diversity is a central topic in the field of evolutionary biology. Through the use of theoretical modeling, progress has been made in understanding the factors that govern the short term fate of diversity. My work expands on this by investigating whether the predictions made by the Levene (1953) and Dempster (1955) models can be extended to include the long term fate of diversity.

Using evolution experiments, I evolved asexual populations of *P. aeruginosa* under two selection regimes in spatially variable environments. A previous study has empirically shown that, in line with theory, diversity within subdivided populations can be stably maintained under soft selection, but not under hard selection (Gallet *et al.* 2018). My work also found that soft selection was indeed better able to maintain diversity when compared to hard selection, and that this diversity was able to persist within the population stably over a longer period of time than hard selection. I found that this maintenance of diversity was driven by negative frequency dependent selection, and that it was underlain by costs of adaptation, a condition that was demonstrated to be crucial (Bell 1997). However, while the conditions that promote the maintenance of diversity persisted under soft selection for longer than under hard selection, I note that there is evidence for the evolution of lower cost resistant genotypes under both forms of selection. This suggests that lower cost resistant genotypes will come to dominate the landscape, and that soft selection will not preserve diversity indefinitely.

Though my work largely draws on the theories of soft and hard selection, there are some distinctions to be made regarding the classical theory and this study. While theory makes predictions on the standing genetic variation within populations, variation in my study stems from the introduction of mutations into the population and its response to ongoing selection. Furthermore, my study also aims to examine the effect of evolution on the predictions made by the models of soft and hard selection. Although the implications from controlled experimental studies are limited when being applied to real populations, they provide a useful intermediary between theory and the real world.

The importance of bridging the gap between theory and the real world becomes more apparent when attempting to use theory to address real life problems. For example, it has been recognized that the models of soft and hard selection provide a useful framework for applied problems that

are concerned with managing the amount of genetic variation within a population. An example would be the management of pests and parasites, where the large amount of pathogen polymorphisms can lead to the evolution of resistance (Mallet 1989; Vale 2013; Leale & Kassen 2018). As my thesis uses a microbial system and uses the evolution of resistance as a co-evolutionary response to antibiotic selection, my work could be used as groundwork for further studies examining the best method to ensure that resistance does not evolve, or does so slowly. An example of such an application of this study would be to model the spread of infectious pathogens within structured environments such as the wards of a hospital.

My thesis has demonstrated that soft selection can better protect polymorphisms within a population than hard selection, and that this is true over both ecological and evolutionary timescales. By empirically testing theory, we can see the strengths and weaknesses of specific models, and from there we create the groundwork upon which we build greater understanding of the world around us.

## **Tables**

**Table 1** : General linear analysis of covariance for comparing the proportion of resistant individuals between soft and hard selection through time.

	<b>Effect size</b>	<b>SE</b>	<b>df</b>	<b>Chisq</b>	<b>P</b>
Intercept	0				
Treatment			2	3021.51	< 0.001
Hard	-1.960	0.0637			
Soft	-2.019	0.0550			
Day			1	1381.26	< 0.001
Treatment : Day			1	19.54	< 0.001
Hard	0.1144	0.004			
Soft	0.0936	0.005			

**Table 2 :** Mixed linear analysis of covariance for comparing the relative fitness of resistant types between day 10 and day 40.

	<b>Effect size</b>	<b>SE</b>	<b>Chisq</b>	<b>df</b>	<b>P</b>
Intercept	1.155	0.0279	41.434	1	< 0.001
Initial			42.0642	1	< 0.001
Day			7.1903	1	0.007
Treatment			0.5255	1	0.468
Initial : Day			11.35	1	< 0.001
Initial : Treatment			2.460	1	0.1167
Day : Treatment			5.448	1	0.0195
Initial : Day : Treatment			6.077	1	0.0136
Hard at day 10	-0.299	0.046			
Hard at day 40	-0.071	0.067			
Soft at day 10	-0.114	0.063			
Soft at day 40	-0.173	0.093			
<b>Random</b>					
Population	0.001890	0.04348			
Residual	0.003569	0.05974			

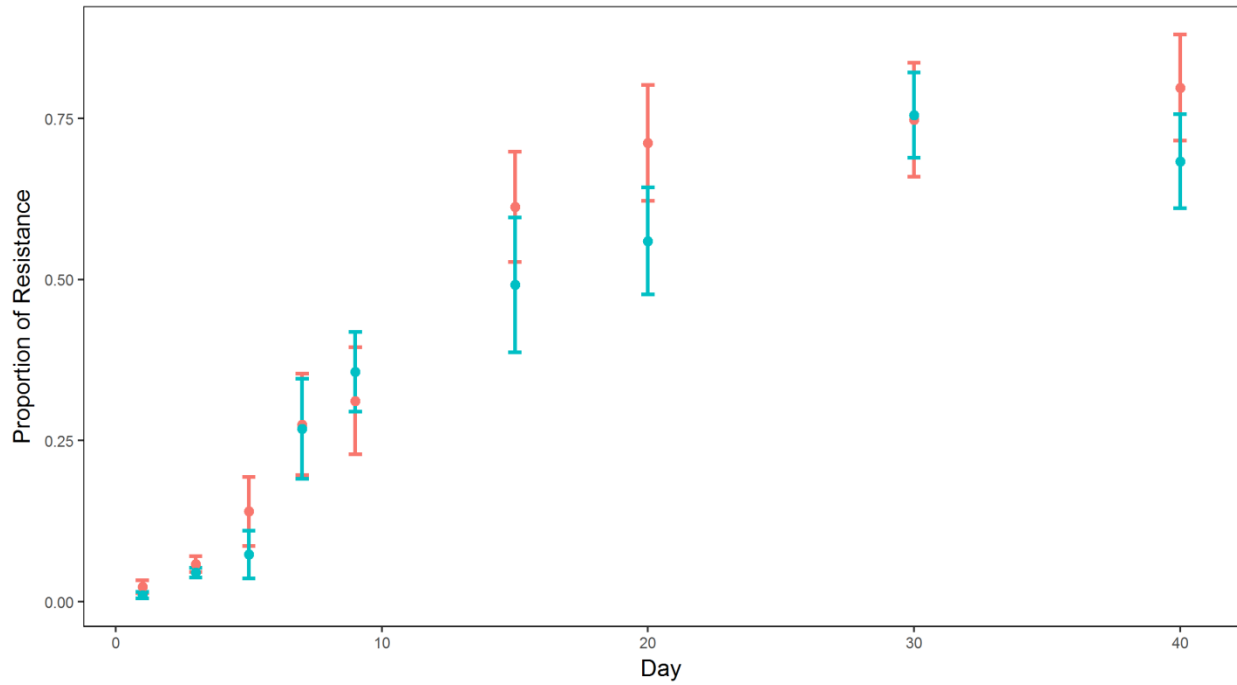
**Table 3 :** Mixed linear analysis of covariance for comparing resistance-growth rate trade-offs between soft and hard selection throughout the experiment. Only populations with resistant and sensitive individuals were included for analysis.

	Effect size	SE	Chisq	df	P
Intercept	1.861	0.1302	204.4554	1	< 0.001
Treatment			2.9946	1	0.0835
LogMIC			24.3592	1	< 0.001
Day			0.6910	3	0.8753
Treatment : LogMIC			0.3962	1	0.5290
Treatment : Day			2.1780	3	0.5363
LogMIC : Day			7.7442	3	0.0516
Treatment : LogMIC : Day			3.4987	3	0.3209
Hard : day 10	-0.3518	0.0683			
Hard : day 20	-0.1681	0.0768			
Hard : day 30	0.0251	0.1051			
Hard : day 40	-0.1607	0.0943			
Soft : day 10	-0.2866	0.0752			
Soft : day 20	-0.1554	0.0838			
Soft : day 30	-0.1249	0.0759			
Soft : day 40	0.04979	0.0745			
<b>Random</b>					
Population	0.0933	0.3054			
Residual	0.2011	0.4484			

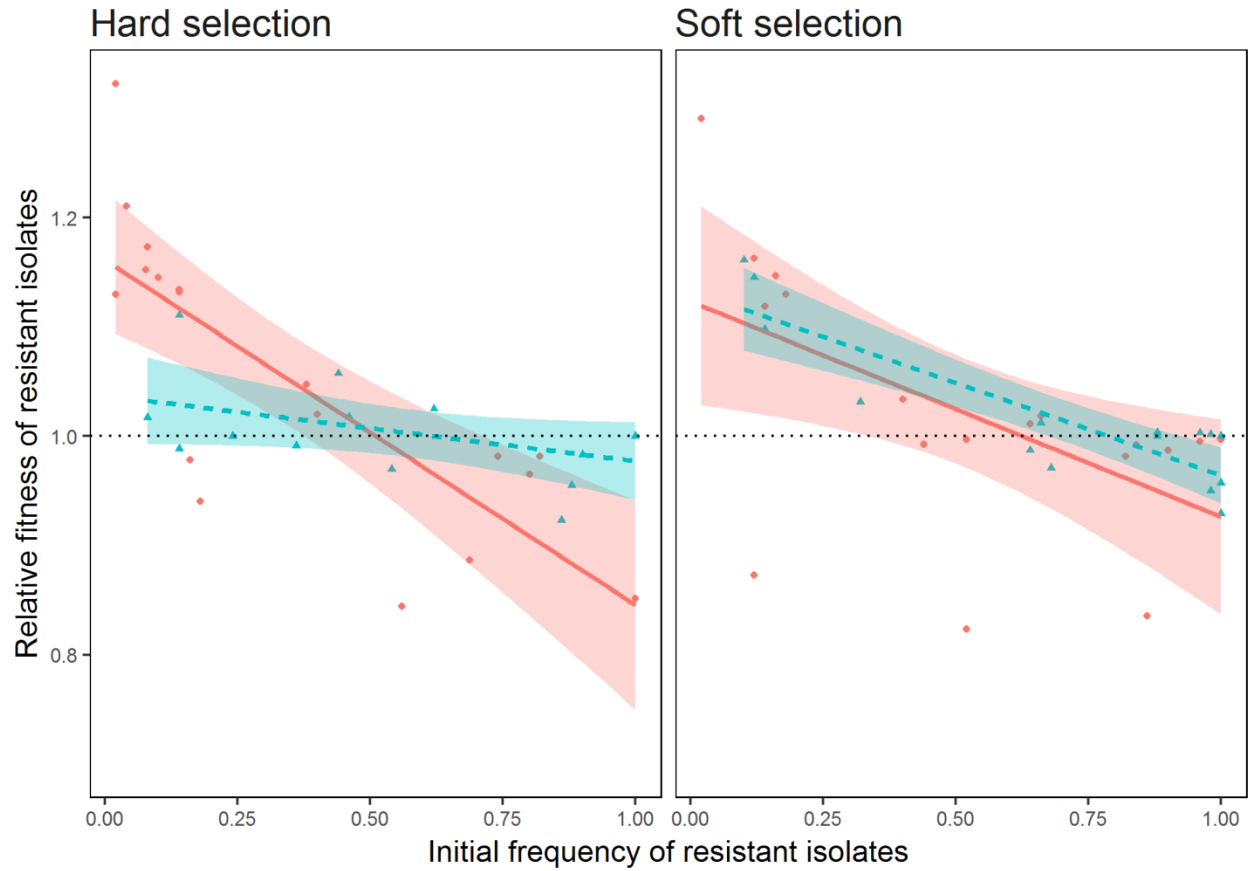
**Table 4 :** Mixed linear analysis of covariance for comparing trade-offs associated with resistance between soft and hard selection throughout the experiment. Only populations with resistant and sensitive individuals were included for analysis.

	<b>Effect size</b>	<b>SE</b>	<b>Chisq</b>	<b>df</b>	<b>P</b>
Intercept	1.861	0.1302	457.0080	1	< 0.001
Day			5.7159	1	0.0168
Treatment			1.4715	1	0.2251
Class			47.7502	1	< 0.001
Day : Treatment			0.0937	1	0.7595
Day : Class			7.6931	1	0.0055
Treatment : Class			8.8505	1	0.0029
Day : Treatment : Class			5.2822	1	0.0215
<b>Random</b>					
Population	0.002633	0.05132			
Residual	0.013601	0.11662			

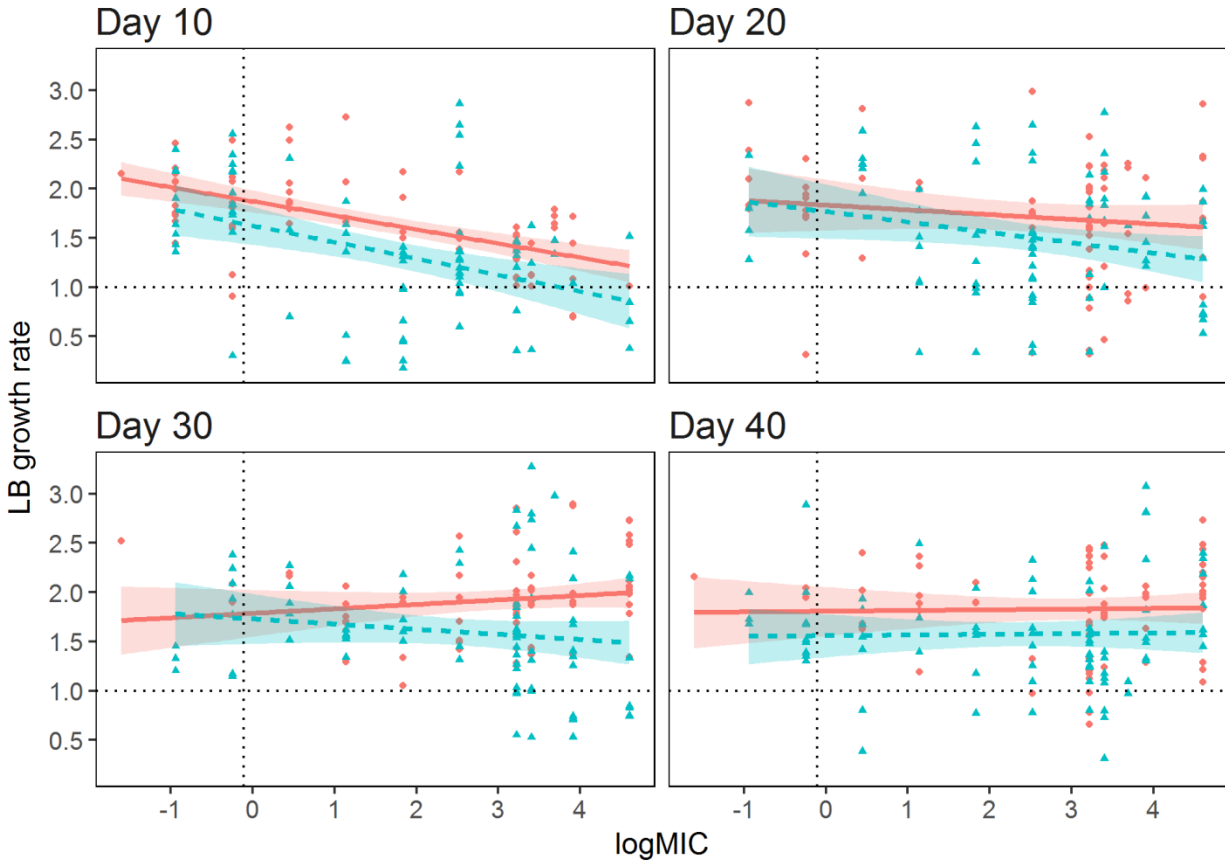
## Figures



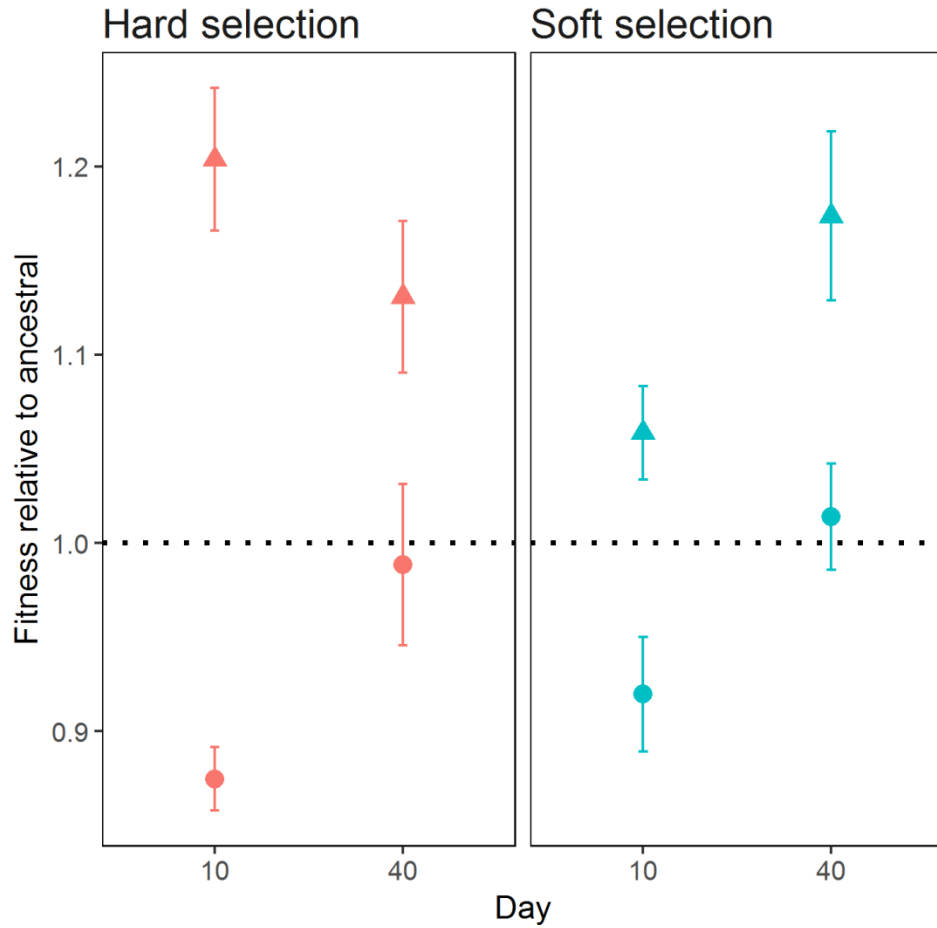
**Figure 1. The proportion of resistant individuals.** Each point represents mean proportion of resistant individuals from either soft selected (blue) or hard selected (red) population replicates (n=12). Error bars represent standard error.



**Figure 2. Negative frequency dependent selection.** Each point represents the relative fitness of a resistant colony relative to a paired sensitive colony when plotted against its initial frequency for day 10 (red) and day 40 (blue). Shaded regions represent standard errors. A signal for negative frequency dependent selection is given by the negative correlation between the relative fitness of resistant isolates and its initial frequency.



**Figure 3. Trade-off between growth rate and log MIC.** Points represent eight isolates taken from each soft selected (blue) or hard selected (red) replicate lines. Growth rates were standardized to the ancestral strains (horizontal dashed line). The vertical dashed line represents the logMIC of the ancestral strain. Shaded regions represent standard error.



**Figure 4. The relative fitness of resistant and sensitive isolates to ancestral.** Each point represents mean relative fitness of resistant (circle) or sensitive (triangle) isolates relative to ancestral strains. Error bars represent standard error. Horizontal dotted line represents fitness of ancestral strains.

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