# Introduction

We study evolution to understand natural diversity; adaptation via natural selection is the cause of complex forms; natural selection acts on genetic diversity; the amount and direction of diversity limits a population to a certain range of possible phenotypes; particularly the additive variance in traits is important because it is heritable;

Additive variance is heritable; explains polygenic traits

At the heart of the evolutionary sciences is the need to understand the natural world’s diversity. Darwin’s (1863; SOURCE) introduction of natural selection some 140 years ago led to increasingly accurate glimpses into the units of evolution, genes, and their movement through a population in response to selection (SOURCE). However, these movements, particularly in a multivariate trait space, become a challenging realm to predict (SOURCE; Lande 1979, 80 etc.). To navigate this space, it is necessary to reduce the predictors of trait trajectories to their principles: how they affect additive genetic variance, the heritable component of trait variability.

Additive genetic variability, VA, is regarded as the most important predictor of a population’s adaptability (Lynch and Lande 1998; Aguirre *et al.* 2014; Careau *et al.* 2015), and hence it’s trajectory through time towards a phenotypic optimum. Although a multitude of stochastic and deterministic processes also contribute to the population’s total trait variability, VA is heritable, and therefore predictive of a population’s trajectory over micro-evolutionary time. The VA of a population determines the phenotypic space that population can explore. Hence, it is predicted that populations with large amounts of VA are best suited to adapting to novel environments (Barton and Charlesworth 1998). Such an example is X. However this is not always the case, standing genetic variation is characterized by a variety of architectural and population-level constraints such as rates of pleiotropy, selection strength, additive effect size, linkage, and deleterious mutation/background selection (SOURCE). For example, under infinitesimal models, selection has a trivial impact on standing variation (Barton 2017).

including genetic drift, selective pressures, additive effect sizes, between- and within-gene interactions, and heritability (SOURCES).

* Natural diversity, population movements in trait space
* Heritable variation
* Stabilising selection, effect on variation/need for variation vs drift
  + Expected to remove variation, mutation alone can’t explain why in natural populations we see so much variation: why?
* Additive effect sizes, effects on variation
* Background selection, effect on variation
* Population genetics expectations of variation under bkg sel, additive effects

# Methods

Using the forward-genetics modelling package SLiM 3.4 (Haller and Messer 2019), I constructed two models to explore a portion of the multivariate parameter space that explains genetic variability in natural populations. These parameters included genome wide recombination rate, the amount of deleterious mutation, the additive effect size distribution, and the selection strength multiplier, (Table 1). The rate of universal pleiotropy, and the amount of mutational covariance between traits was also varied across models but was not considered for analysis. Among these models, multiple conditions and assumptions are shared.

## Common model elements

Both of my experimental models consisted of a SLiM 3.4 model simulating a Wright-Fisher population of 8000 diploid individuals evolving over 100,000 generations (with an additional 50,000 generations of burn-in (more information in figure S1 – heterozygosity figure from burn-in test). Each individual is characterized by 8 traits, controlled by 100 loci each. Each trait has an identical effect on fitness, forming a ‘mega-trait’ with varying variance-covariance structures depending on pleiotropy rates. Each locus is assumed to have identical length, and each base pair within it is assumed to be mutationally independent, an assumption supported by a study by Thornton (2019), which found that within-locus differences in linkage had no average effect on either genetic variance or the mean trait value, indicating within-locus independence. In addition, the average number of base pairs per locus is highly conserved within eukaryotes (Xu *et al.* 2006), indicating that the assumption of equal gene length is not too far-fetched. The mutation is modelled as occurring at an arbitrary position within the locus (or its regulatory regions) and is of arbitrary form. The effect of the mutation on chromosomal structure (e.g. effects of deletions, insertions etc.) is not explicitly modelled, but is implied via their effect on fitness and/or the trait. Mutations are assumed to be completely additive in effect, with no dominance or epistatic interactions. All loci are assumed to be on the same chromosome, with genetic distance being determined by the recombination rate parameter, r (Table 1). Both models have a genome-wide germline mutation rate of 8.045x10-6 per locus per generation, based on an average of five groups of eukaryotes (Aston *et al.* 2017).

The chosen effective population size, Ne = 8000, was chosen to compromise between computational performance and the effect of genetic drift on populations under stabilizing selection. This value results in weak genetic drift in comparison with the strength of selection (under strong selection pressures), and appropriate standing genetic variation following burn-in to allow for adaptation (Lynch and Lande 1998).

Mutational effects on trait values were sampled from a normal distribution,

where λ is the additive effect size (Table 1). In the case of pleiotropy, a multivariate normal distribution was used, where n = 8, and

where **Σ** is a covariance matrix with diagonal values equal to λ and non-diagonals pulled from a normal distribution:

where is the parameter value of mutation correlation. **Σ** was ensured to be positive definite by multiplication with its transpose.

Non-trait deleterious mutations had fitness effects sampled from a gamma distribution with

Where and (SLiM Manual). This describes a distribution of fairly weak deleterious mutations on average.

All models were subject to 50,000 generations of burn-in, where mutations accumulate until the population reaches mutation-drift equilibrium. This is tracked as heterozygosity through the simulation, where mutation-drift equilibrium occurs when:

where µ represents the per-locus mutation rate per generation (Kimura and Crow 1964). A population at equilibrium was assumed sufficiently burnt in. Trials indicated that 50,000 generations was sufficient for our population size (FIGURE S1: Plot of heterozygosity). Deleterious mutation (δ) lowered the value of away from expectation in initial burn-in tests, however an alternative equilibrium was reached, satisfying the requirements of burn-in (Figure S1).

## Model specific characteristics

After reaching equilibrium, populations evolved for 100,000 generations of neutral drift or stabilizing selection, depending on the treatment. Neutral drift entailed no change from the properties of the burn-in, whereas stabilizing selection imposed a fitness function on phenotypes, invoking a multivariate optimum a fixed distance from the population mean phenotype post-burn-in. The position of the optimum is defined as:

Where is the vector of phenotype means, is the per-locus, per-generation mutation rate, , is the number of mutational steps to reach the optimum, and is the number of generations of burn-in. For our purposes, and .

The fitness of an individual in the population was defined as:

Where s represents strength of selection, represents the gradient of the selection curve, n is the number of traits, and xn is the phenotype for trait n. For my experiments, s was fixed at s = 0.9, ensuring minimum fitness was 0.1, and maximum fitness was 1. This results in individuals at the optimum being at most ten times as fit as those infinitely far from the optimum. This value differs depending on, which adjusts the realized fitness gradient.

## Model Parameterization

Five parameters were shared between models, with a sixth for testing selection (Table 1). These were sampled using a Latin hypercube sampling design, with 1024 parameter combinations testing the null model, and 256 for the selection model. These samples were generated using the R packages ‘DoE.Wrapper’ and ‘LHS’, using the maximin algorithm (Melo *et al.* 2015; R Developmental Core Team 2019). Each model was repeated 100 times, using 100 seed values fed to SLiM. These seeds were randomly sampled from a uniform distribution of the total range of unsigned 32 bit integers (1 to 232 – 1) using the runif() function in base R (R Developmental Core Team 2019). The array of parameter combinations and replicates was processed across 1152 cores on the University of Queensland’s Tinaroo high performance computing (HPC) system, using embedded Nimrod scripts to feed parameter/seed combinations to individual SLiM processes.

## Analysis

Despite not all data conforming to normality, no data was transformed owing to the large sample sizes. Previous work into the robustness of regression modelling, t-tests, and F-tests have shown that departures from normality can usually still provide reliable estimates, provided the number of observations is large enough that coefficient estimates are approximately normally distributed due to the central limit theorem (Lumley *et al.* 2002). This was verified with diagnostic tools in the R package “jtools” (Long 2020). Heteroscedasticity was accounted for using Eicker-Hubert-White (EHW) robust standard errors, although owing to the large sample size, this adjustment had minimal effect on t-statistics (Eicker 1967; Huber 1967; White 1980).

For analysis, each parameter was grouped into three categories: low, medium, and high, with each bin containing a third of the total data. was the exception to this, with a fourth bin, null, describing the neutral models with no value at all. Across all analyses, means of responses were compared at the final generation of the simulation (100,000) and variances of responses over time (from generations 50,000 to 100,000). Trait variances and covariances were pooled and averaged to form a ‘mega-trait’ average variance and covariance, since traits were functionally identical. These were compared across groups using a robust multiple regression with EHW standard errors, followed by estimated marginal mean comparisons between groups using the ‘emmeans’ package in R (R Developmental Core Team 2019; Long 2020).

In addition, I computed the population mean Euclidean distance from the optimum for each replicate and model:

comparing these distances with multiple regression, again with EHW robust standard errors. Contrasts between bins were also compared with estimated marginal means.

# Results

## Variance and covariance

Although the residuals of groups strayed from normality, variance was homoscedastic and the number of observations (102400) led to normality of coefficient estimates through the central limit theorem, providing some robustness from the effects of non-normality (Lumley *et al.* 2002).

To compare the effects of parameters on variance-covariance structure, I computed mean variances and covariances across traits, describing the total variance/covariance in the ‘mega-trait’ space.

Variance showed a cyclic behavior under all models around an equilibrium which differed depending on parameter combinations. To explore the nature of this variation, I fit a linear model to the variance of the mega-trait variance over a period of 50,000 generations (from generation 50,000 to 100,000, where all models had reached their local equilibria). This model included main effects of all parameters, as well as pairwise interactions and a three-way interaction between deleterious mutation rate, recombination, and additive effect size. Significant main effects for deleterious mutation rate, recombination rate, and additive effect size on variance of variance over time were observed, along with significant interactions between deleterious mutation and recombination, deleterious mutation and additive effect size, recombination rate and additive effect size, pleiotropy rate and additive effect size, additive effect size and selection strength, and between deleterious mutation, recombination rate, and additive effect size (F16, 1261 = 90.76, p < 0.0001, Adjusted R2 = 0.6684).

Per unit increase, deleterious mutation rate increased the variance of mega-trait variance over time by 16550 ± 3185 units (t1261 = 5.1971, p < 0.0001). Increasing recombination rate also increased variance of variance by 0.9694 ± 0.2667 units per 1x10-8 increase in recombination rate (t1261 = 3.6343, p = 0.0003). Additive effect size increased variance of variance by 6371 ± 610.4 units per unit of effect size (t1261 = 10.437, p < 0.0001). Deleterious mutation reduced recombination’s effect on variance of variance by 1.177 ± 0.3361 units for every 10-8 units of recombination and every unit of deleterious mutation (t1261 = -3.5016, p = 0.0005). Deleterious mutation also interacted with additive effect size, reducing additive effect size’s increase on variance of variance by 6975 ± 899.6 units (t1261 = -7.7529, p < 0.0001). Recombination and pleiotropy rate both decreased additive effect size’s per-unit increase of variance of variance, by 0.3815 ± 0.07665 units per 10-8 units of recombination (t1261 = -4.9771, p < 0.0001), and 1238 ± 374.2 units (t1261 = -3.309, p = 0.001), respectively. In addition, deleterious mutation significantly interacted with the interaction between recombination and additive effect size. The reduction of variance of variance caused by increasing additive effect size while increasing recombination rate was further magnified by increasing deleterious mutation rate by 0.5001 ± 0.1102 units per unit of deleterious mutation rate and additive effect size and per 10-8 units of recombination rate (t1261 = 4.5398, p < 0.0001).

Deleterious mutation may increase variance of trait variance, but the effect of this is dependent on additive effect size – greater increases under greater effect sizes. Recombination can somewhat reduce this interaction by splitting deleterious mutations from additive effects, decreasing the effect of deleterious mutation on variance of variance under larger additive effects (relative to lower levels of recom).

Variance tended to… with increasing deleterious mutation, locisigma, rwide, selection strength. Null vs sel

Covariance tended to… with selection strength.

## Distances from the optimum

Euclidean distances from the optimum were also compared at the end of the simulation. The resulting linear model, containing main effects, pairwise interactions, and the three-way interaction between deleterious mutation rate, recombination rate, and additive effect size, showed significant trends in regards to changes in all main effects, and all interactions barring those between recombination and selection strength, and recombination and additive effect size (F16, 127983 = 21190, p < 0.0001, Adjusted R2 = 0.7496). Increasing recombination rate led to increases in distance from the optimum of 0.0009 ± 0.0003 units for every 10-8 increase in recombination rate (t127983 = 3.747, p = 0.0002; FIGURE). Increases in additive effect size led to increases of distance of 70.05 ± 0.4576 units (t127983 = 153.093, p < 0.0001; FIGURE). Increasing pleiotropy rate had a similar effect on distance from the optimum, with every 10% increase in pleiotropy rate resulting in 19.87 ± 0.5047 units of distance (t127983 = 39.364, p < 0.0001; FIGURE). Increasing selection strength increased the distance from the optimum by 0.05502 ± 0.00428 units for each unit of (t127983 = -12.862, p < 0.0001).

SELECTION STRENGTH BIAS: 0 = NULL, 10 = STRONG 1000 = WEAK

I think this is biasing the linear model

Significant interactions were observed between all pairwise combinations of parameters barring recombination and additive effect size.

Under strong selection, increasing background selection from low to high resulted in an average decrease in distance from the optimum of 136.5 ± 2.94 units (t25471 = 46.467, p < 0.0001). Under intermediate selection, recombination rate interacted with deleterious mutation, reducing the effect of deleterious mutation on distance. Under intermediate selection and low recombination, when increasing deleterious mutation, distance from the optimum declined 247.80 ± 7.68 units (t25471 = 32.248, p < 0.0001). Under high recombination rates, this decline was 92.26 ± 5.10 units (t25471 = 18.077, p < 0.0001). This drop of 155.5 ± 9.16 units between contrasts was highly significant (t25471 = 16.983, p < 0.0001). A similar effect was seen under weak selection, however the contrast was marginally insignificant (decrease of 28.3 ± 10.01 units between low and high recombination when deleterious mutation increased from low to high; t25471 = 2.830, p = 0.0528).

Similarly to variance, the distance to the optimum also showed a cyclic behavior (Figure 2). To explore the variation in these cycles, the variance in Euclidean distance over generations 100,000 – 150,000 was compared between models. A linear model fit to the main effects and pairwise interactions of all parameters showed statistically significant effects for deleterious mutation rate, pleiotropy rate, additive effect size, and selection strength, as well as interactions between deleterious mutation rate and additive effect size, pleiotropy rate and additive effect size, and deleterious mutation rate and selection strength (F16, 1263 = 383.3, p < 0.0001, Adjusted R2 = 0.873).

Increasing deleterious mutation overall increased variance in distance over time by 4209 ± 970 units (t1263 = 4.3391, p < 0.0001). Similarly, increasing pleiotropy rate, additive effect size, and decreasing selection strength increased variance in distance over time by 5917 ± 1771 units (t1263 = 3.3415, p = 0.0009), 3493 ± 122.5 units (t1263 = 28.5065, p < 0.0001), and 7.109 ± 1.273 (t1263 = 5.5841, p < 0.0001) units per unit change, respectively. Recombination had no significant effect on variation in distance to the optimum over time. Additive effect size decreased the effect of background selection by 2798 ± 133.9 units (t1263 = -20.8863, p < 0.0001) per unit of effect size. A similar effect was observed with pleiotropy rate, with additive effect size decreasing the effect of pleiotropy rate on variance in distance to the optimum by 761 ± 216 units (t1263 = -3.5228, p = 0.0004). Deleterious mutation also interacted with selection strength, increasing the effect of selection strength by 3.89 ± 1.672 units (t1263 = -2.3265, p = 0.0202).

# Discussion

In theoretical quantitative genetics, much debate is had over which particular models best describe the maintenance of variation in the presence of stabilizing selection over time. Selection is able to retain variation, particularly in large populations where drift is weak, and scenarios where balancing selection creates a non-linear fitness landscape, however the extent of this differs depending on many factors, including selection strength, genetic architectures, epistatic and dominant interactions, and the strength of selection relative to mutation (Walsh and Lynch 2018). Understanding the relative strength of selection to mutation has led to two distinct approximations of expected distributions of allelic effects. When mutation is much stronger than selection, Kimura (**1965a**) and Fleming’s (**1979**) Gaussian approximation holds true, whereas when the opposite occurs, Turelli’s (**1984**) house of cards approximation is more accurate. This distinction between models is arbitrarily granular, mostly for analytical viability. Computational methods allow for a continuous exploration of this space of models.

# Snippets

Underpinning this model is the continuum of alleles model of allelic effects, suggesting large numbers of alleles at many loci forming a continuous distribution of effect sizes, usually Normal in shape (Lande).

Pleiotropy fundamentally alters the signatures of HCA vs Gaussian approximation in COA so they approach each other - other parameters may as well?

Most effort in understanding stabilizing selection has focused on assuming either a Gaussian (as in this paper) or quadratic fitness function

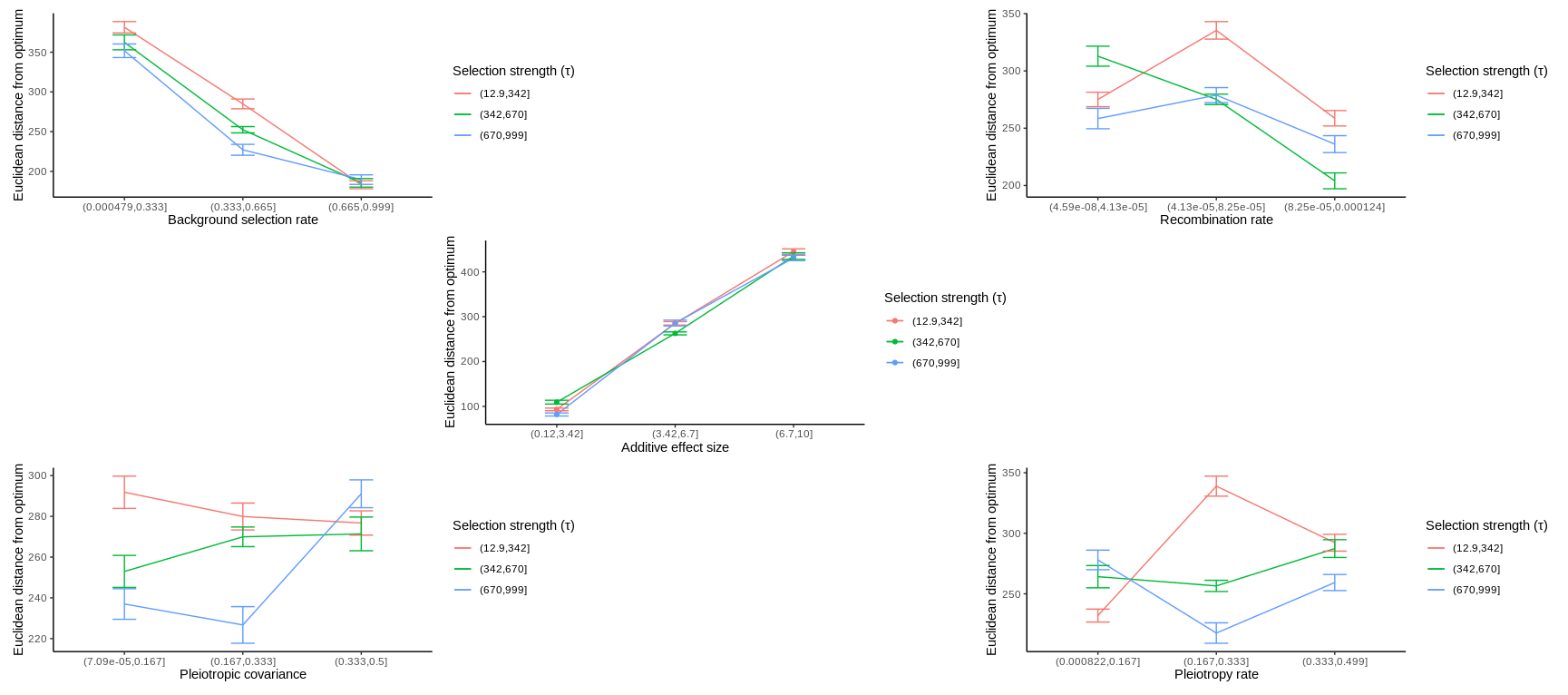


Figure 1 – Euclidean distance from the optimum by parameter and selection strength

Table 1: Model parameters for both null and stabilizing selection models. The range of values is based on literature, but values are adjusted to be practical for the time of the experiment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Symbol | Range | Description | Source(s) |
| Genome wide recombination rate | r | 0 to 1.241x10-4 per locus | The singular recombination rate used across the entire simulated genome. | Stapley et al. 2017 |
| Background selection rate | δ | 0 to 1 | The number of non-trait, deleterious mutations that occur relative to trait mutations. |  |
| Rate of universal pleiotropy | ϖ | 0 to 0.5 | The proportion of trait mutations that affect all traits rather than a single trait. While 100 loci control a trait independently by default, this may be changed by this parameter. However ratios of loci affecting each trait will remain constant, especially across multiple replicates. | Chesmore et al. 2017; |
| Mutational pleiotropic correlation | m | 0 to 0.5 | The mutational correlation between additive effects of pleiotropic mutations determines the similarity of trait effects between traits for the same pleiotropic mutation. |  |
| Additive effect size | λ | 0.1 to 10 | Additive effect size controls the variance of trait effect size around mean 0, so that N(0, λ). | Albert et al. 2008; |
| Selection strength (selection model only) |  | 10 to 10000 | The parameter that controls the curve of the fitness function (eq. 3), with higher values resulting in a smaller difference in fitness between trait-differing individuals. |  |

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