# 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 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, additive genetic variance is heritable, and hence is the component that can be

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

* Importance of genetic variability for adaptation
* Redundancy and genetic pathways to polygenic adaptation
* Additive models of quantitative genetics, Fisher’s geometric model
* Connection between quantitative and population genetics approaches (Geometric model, interaction of traditionally mutation-driven pop gen features (e.g. deleterious mutation, allele frequencies, selective sweeps) with more traits)
* Effects of deleterious mutation on genetic variability, constraining adaptation
* Recombination and linkage in the context of creating largely deleterious haplotypes with non-trait affecting deleterious mutations
* Effects of pleiotropy and the cost of complexity
* Deleterious mutation and pleiotropy as constraints on adaptation – expectations under geometric model
* Theories of adaptation in quantitative genetics – stabilising, disruptive, directional, squashed stabilising
* SLiM as a tool to computationally study these effects over long time scales
* Introduction of aims – to quantify the effects of deleterious mutation and pleiotropy on neutral evolution in an intermediate-sized population & to quantify the effects of del muts and pleio on adaptation to an intermediate optimum
* Novelty: effects of deleterious mutation and recombination on multiple traits – do they behave the same way as with just one or two traits?

# 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 rate of universal pleiotropy, the mutational correlation between trait effects from a single pleiotropic mutation, the additive effect size distribution, and the selection strength multiplier, (Table 1). 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, unless a pleiotropic treatment is applied which will randomly reduce this by an approximately uniform amount per trait (further detail below). Each locus is assumed to have identical length, and each base pair within it is assumed to be mutationally independent. This behavior seems reasonable, as a study by Thornton (2019) 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, 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.

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 192 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.

## Null model analysis

To compare null models generally, I compared heterozygosities at generation 150,000 using a linear mixed effects model, with the SLiM seed value as a random effect. 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 (Lumley *et al.* 2002). This was verified with diagnostic tools in the R package “jtools” (Long 2020), along with the homoscedasticity of variance, which was good across all data.

To compare the complete range of variance and covariance via principal components analysis (PCA), I binned each parameter into three equidistant factor levels, representing high, medium, and low factor levels for each variable. From the population variances and covariances, I extracted **G** matrices and performed PCA on them, constructing **G** ellipses from the first two traits and principal components for each parameter factor level. Using five-way type-III ANOVAs (comparing only first-order interactions between predictor variables), I compared the areas of each ellipse, the ratio of the major and minor axes of variation, and the angle of rotation of the ellipse around its center (the two-trait mean). I then performed post-hoc least-squares means tests (adjusted for multiple comparisons with Tukey’s correction) to determine which groups were significantly different.

To describe the total structure of differences between **G** matrices across all eight traits, I used relative PCA, comparing differences in variance-covariance structure both within and between levels of each predictor variable. This was done using the ‘vcvComp’ package for R (Le Maître and Mitteroecker 2019). Relative principal components analysis produces the generalized variances between two tested models, which is the product of all relative eigenvalues (Le Maître and Mitteroecker 2019). This is equivalent to the ratio of the determinants of the two covariance matrices (or models) being compared. The log generalized variance is a useful metric for comparing the magnitude of variation across all traits between the two groups (Le Maître and Mitteroecker 2019). I sampled 128 models of the total 1024 for 812,800 pairwise comparisons between models (8128 comparisons replicated 100 times), computing a relative PCA for each comparison.

Using a similar methodology to my ellipse analysis, I used five-way type-III ANOVAs to compare log generalized variances between groups. I then computed post-hoc least-squares means tests, adjusting for multiple comparisons with Tukey’s correction. I then compared the distributions of log generalized variances between groups using Kolmogorov-Smirnov tests, and the variance between groups with a five-way type III ANOVA, followed again by least-squares means post-hoc tests.

## Selection model analysis

I repeated the above analyses on the selection model, this time including the sixth predictor, , in statistical models. Heterozygosity analysis was excluded due to lost data. I grouped the five predictors into bins as with the null model, but included selection strength as another parameter to compare these parameters against. In addition, I computed the population mean distance from the optimum for each replicate and model, comparing these distances with another type III ANOVA.

## Null/selection comparisons

To compare null models to selection models, eigentensor analysis was used to compare groups of **G** matrices with similar parameter values…

# Results

## Null model

I first analysed the effects of background selection, recombination rate, pleiotropic covariance, rate of pleiotropy, and additive effect size (along with their first-order interactions) on heterozygosity with a linear mixed effects model. This model explained 81.7% of total variation. I found that increasing background selection reduced heterozygosity, (β = -0.137, t102384 = -192.932, p < 0.0001), recombination rate increased it (β = 155.5, t102384 = 27.016, p < 0.0001), and recombination alleviated some of the effects of background selection on heterozygosity via interaction (β = 122.9, t102384 = 22.263 , p < 0.0001). 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 **G** ellipses of traits 1 and 2, comparing ellipse area major-minor axis ratios, and angles of rotation around the center with a series of type III ANOVAs (Figure 1).

Ellipse area tended to decrease with increasing deleterious mutation, however the extent to which this occurred depended on other variables. For example, with low levels of recombination, increasing deleterious mutation rates from the lowest to highest levels resulted in a decrease in ellipse area of 1183 ± 97.6 units2 (t102349 = 12.112, p < 0.0001). With high recombination, the same change in deleterious mutation saw a decrease in ellipse area of 663 ± 100.4 units2 (t102349 = 6.609, p < 0.0001). The effects of increases in recombination rate also depended on other variables. Under low rates of pleiotropy, changes in recombination rate had no significant effect on **G** ellipse area (t102349 = -1.057, p = 0.5408). Under high pleiotropy rates, increases in recombination decreased ellipse area by 368.5 ± 99.4 units2 (t103249 = 3.707, p = 0.0006). With intermediate levels of pleiotropy, increases in recombination resulted in increases to ellipse area by 279.2 ± 100.2 units2 (t103249 = -2.776, p = 0.0152).

The ratios of major and minor axes of the **G** ellipses remained mostly stable across treatments. Although significant differences were found, the magnitude of these was less than 0.1 degrees.

The angles of rotation of **G** ellipses around their centroid also differed between groups. Increasing deleterious mutation in both high and low recombination groups led to similar counter-clockwise rotations around the mean: 36.27 ± 1.1 degrees (t103249 = -32.886, p < 0.0001), and 37.3 ± 1.07 degrees (t103249 = -34.761, p < 0.0001), respectively. Intermediate levels of recombination reduced this rotation to 29.28 ± 1.12 degrees (t102349 = -26.072, p < 0.0001). When under low levels of pleiotropy, increasing deleterious mutation led to counter-clockwise rotations of 26.51 ± 1.10 degrees (t102349 = -24.026, p < 0.0001), whereas high rates of pleiotropy led to an increase of this rotation to 48.88 ± 1.11 degrees (t102349 = -44.148, p < 0.0001). A similar effect is seen when increasing recombination rate, which has little effect individually on rotation (Figure 1). However, under high pleiotropy there is a small counter-clockwise rotation of 7.049 ± 1.09 degrees (t102349 = -6.496, p < 0.0001).

**A lot of these interactions are significant, so I’ll probably put them all in a table and only talk about the interesting ones.**

To compare the effects of the parameters on total variance-covariance structure, I used relative PCA, comparing pairs of models in the same bin against those the furthest apart (i.e. relative PCA between two models with very similar values for a given parameter, compared against relative PCA between two models with maximum difference in values for a given parameter). Mean values of log generalized variance were compared between these two groups for each parameter (Figure 2). Large discrepancies in deleterious mutation between pairs resulted in higher log generalized variance (t17626 = -23.433, p < 0.0001), with a mean difference of 2.539 (**log generalized variance – not sure how to interpret what this number actually means in a quantitative sense**). The same was true for rate of pleiotropy (t24740 = -5.709, p < 0.0001; = 0.61), pleiotropic covariance (t14700 = -7.017, p < 0.0001; = 0.925), and additive effect size (t17295 = -19.961, p < 0.0001; = 2.356). Recombination rate differed from the other results, with log generalized variance decreasing with increasing differences between paired models (t7275.4 = 9.405, p < 0.0001; = -1.51). As well as differences in means, distributions of log generalized variances also differed significantly.

I used two-sample Kolmogorov-Smirnov tests to assess differences between the distributions of log generalized variance between similar- and distinct-parameter pairs (Figure 3). All distributions were significantly different (Table 2). Comparisons between models with large differences in deleterious mutation frequency showed considerable increases in frequencies of log generalized variances around zero, with bimodal peaks on either side of zero (Figure 2). Comparisons between models with large differences in either pleiotropy rates or additive effect size also led to multimodal distributions, with a bottleneck effect appearing on either side of the center, with compression of the tails (Figure 2). Large differences in recombination similarly compressed the range of variation in log generalized variance between models, with a strong bottleneck appearing below zero log generalized variance. Large differences in pleiotropic covariance led to more subtle patterns, where small bottlenecks and tail compression were visible, but to a much lesser extent than with the other parameters.

## Selection model

To compare the effects of parameters on variance-covariance structure, I computed **G** ellipses of traits 1 and 2, comparing ellipse area, major-minor axis ratios, and angles of rotation around the center (Figure 4). Within all three responses, interactions between variables were highly significant.

The area of the ellipse was greatly affected by changes in all parameters. Under strong selection, increasing background selection from low to high levels resulted in a decrease of 1671 ± 210 units2 in area (t25527 = 7.962, p < .0001). Under weak selection however, the same increase in background selection led to larger decrease of 5002 ± 223 units2 (t25527 = 22.467, p < 0.0001). Increasing recombination rate in both cases decreased ellipse area, however more-so at high selection strength (high selection: 2665.3 ± 209 units2 of change; t25527 = 12.762, p < 0.0001; low selection: 3781 ± 227 units2; t25527 = 16.622, p < 0.0001). Increasing the rate of pleiotropy increases **G** ellipse area, under both high and low selection strengths; this effect is stronger under high selection strengths (high selection: 4000.1 ± 212 units2; t25527 = -18.866, p < 0.0003; low selection: 1426.7 ± 221 t25527 = -6.447, p < 0.0001).

The angle of rotation around the centroid significantly differed between many groups, with strong interactions. Under strong selection, increasing background selection led to a counter-clockwise rotation of 13.56 ± 1.99 degrees (t25527= -6.797, p < 0.0001). Under weak selection, there was no significant change in rotation when background selection was increased from low to high. When recombination is increased, selection has a similar effect on rotation. Under strong selection, increases in recombination lead to clockwise rotations of 39.94 ± 1.98 degrees (t25527 = 20.128, p < 0.0001), whereas under weak selection this effect is reduced to 23.79 ± 2.16 degrees (t25527= 11.008, p < 0.0001). When increasing the rate of pleiotropy, under strong selection, there is a counter-clockwise rotation of 16.36 ± 2.01 degrees (t25527 = -8.119, p < 0.0001). Under weak selection however, the mean rotation is increased to 20.72 ± 2.10 degrees in the counter-clockwise direction (t25527 = -9.856, p < 0.0001).

Major-minor axis ratios were fairly static, with significant differences being less than 0.1 in magnitude.

**Almost all comparisons are significant: I think I’ll put the total results in a table and just write out the most interesting ones that I’ll talk about in my discussion.**

I repeated the relative PCA analysis for the selection model, considering interactions between selection strength and the other parameters. Under strong selection, large differences between models being compared led to a decrease of 0.4594 ± 0.165 log units of generalized variance (t812727 = 2.778, p = 0.0151) relative to a pair of similar-background-selection-rate models, regardless of what that rate was. Under weak selection, there was no effect of the similarity of models in a pair on log generalized variance (t812727 = 0.079, p = 0.9966). Differences in recombination rate between pairs of models led to differences in mean log generalized variance under both weak and strong selection (strong selection: 2.551 ± 0.165 log units; t812727 = -15.481, p < 0.0001; weak selection: 1.555 ± 0.245 log units; t812727 = -6.336, p < 0.0001). A similar effect was seen with pleiotropic covariance divergence between pairs; under high selection, shifting from a similar pair to a divergent pair resulted in a decrease of 0.895 ± 0.168 log units of generalized variance (t812727 = 5.331, p < 0.0001). Under low selection, this decrease was 3.432 ± 0.251 units (t812727 = 13.647, p < 0.0001). Differences in pleiotropy rate divergence between models had no effect on log generalized variance regardless of selection strength, but this was only true in when comparing extremes. Comparisons between models with medium amounts of divergence and either little divergence or maximum divergence led to significant changes in log generalised variance under both strong and weak selection, but not intermediate levels (high selection strength, low to medium 1.152 ± 0.13, t812727 = -8.876, p < 0.0001; medium to high -1.254 ± 0.18, t812727 = 6.984, p < 0.0001; low selection strength, low to medium -0.829 ± 0.222, t812727 = 3.732, p = 0.0006). The exception to this was under weak selection, comparisons between medium-pleiotropy and high-pleiotropy led to a marginally insignificant difference in log generalized variance (0.636 ± 0.279, t812727 = -2.278, p = 0.0589).

# Snippets

, rate of pleiotropy (β = 0.012, p < 0.0001), and additive effect size (β = 0.00022, p = 0.0023) on heterozygosity, as well as significant interactions. The most biologically meaningful of these interactions included interactions between deleterious mutation and recombination rate (β = 122.9, p < 0.0001).

I then analysed the effects of the parameters on variance and covariance, again using a linear mixed effects model, and choosing to focus on the variance of trait 1 and covariance between traits 1 and 2 as a proxy for all of the variance and covariance terms. I did a more complete analysis of the total variance-covariance structure with PCA and relative PCA later on. **Nonsense linear regression results – by adding other factors, delmu increases variation (?), despite the actual data not fitting that trend at all, but by excluding the other factors delmu decreases variation?**

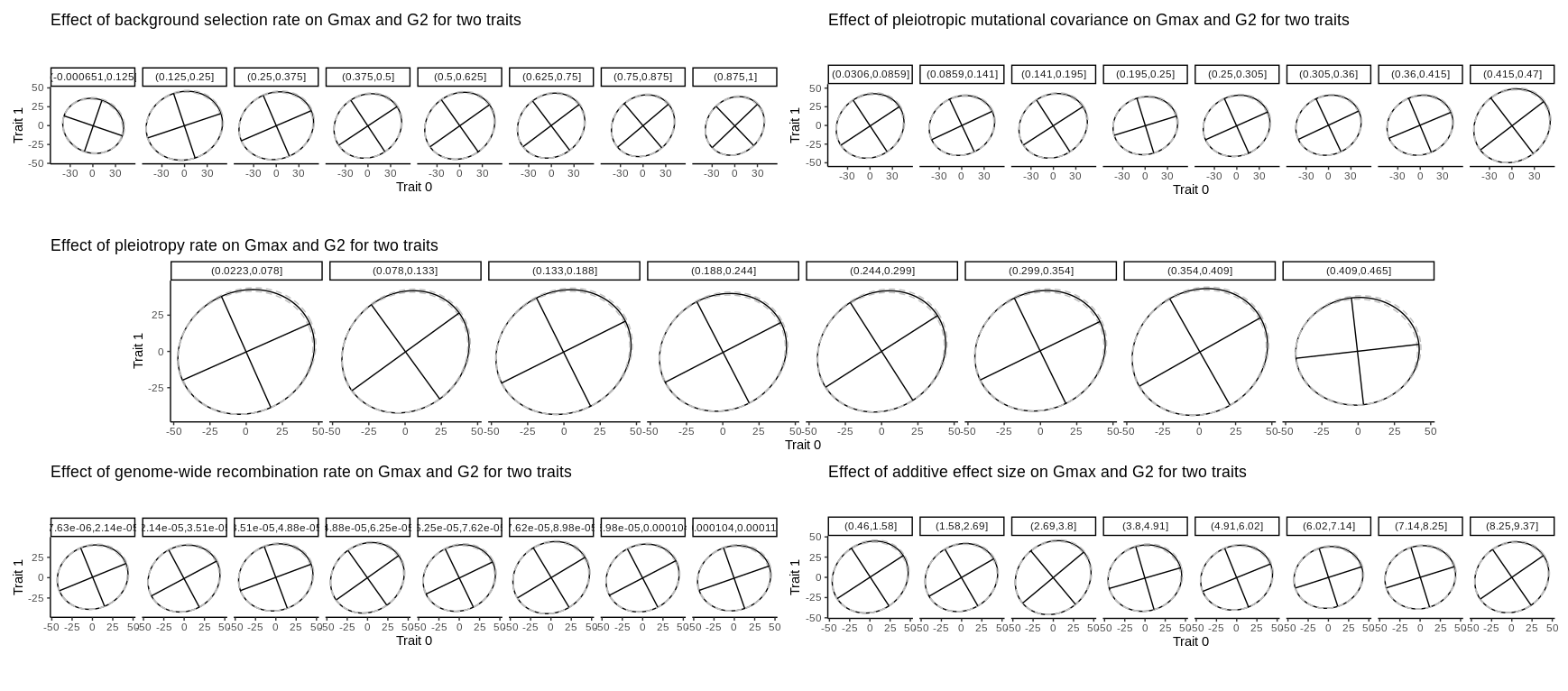


Figure 1 – **G** ellipses by the parameters. Parameter values increase from left to right.

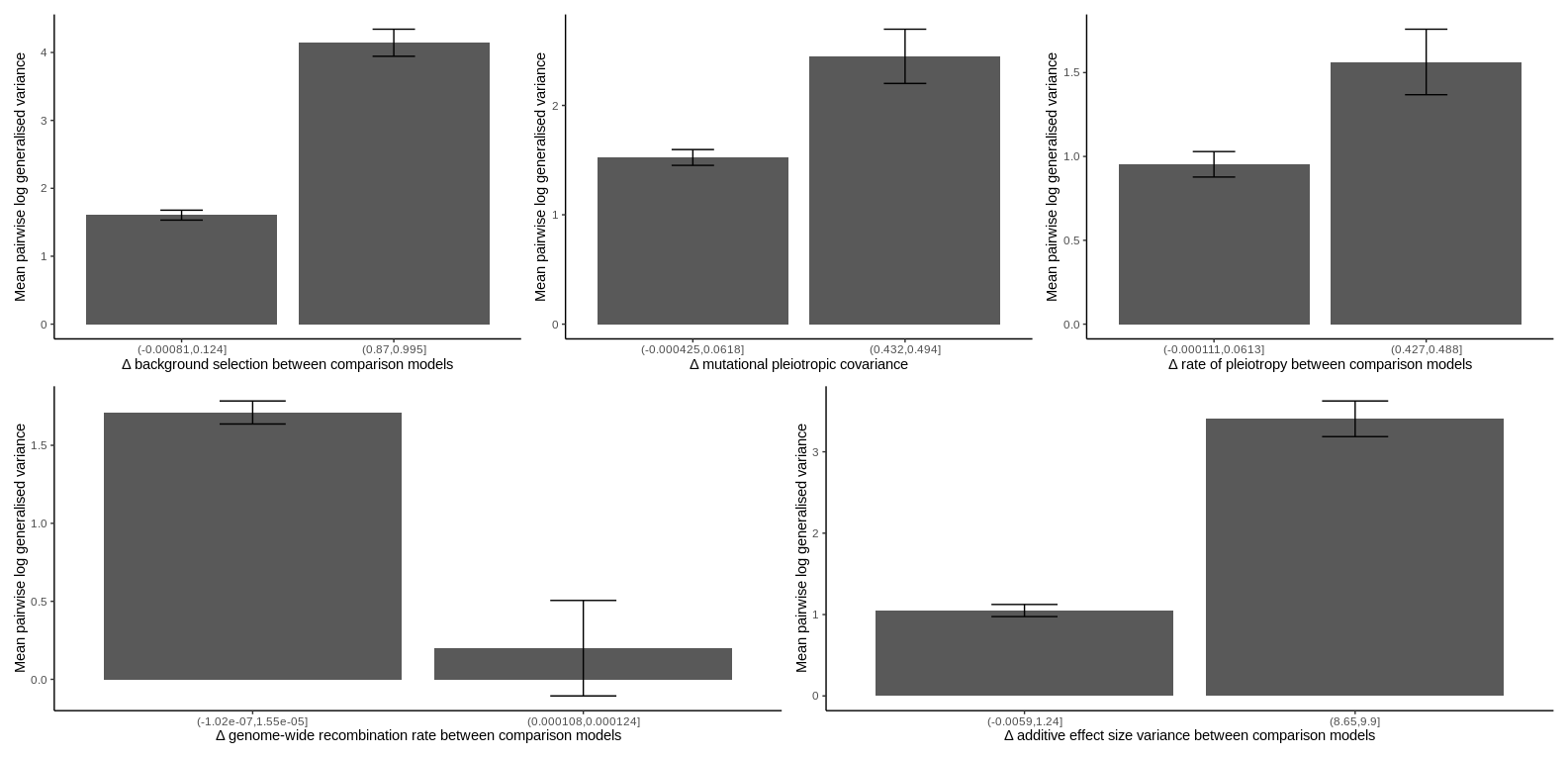
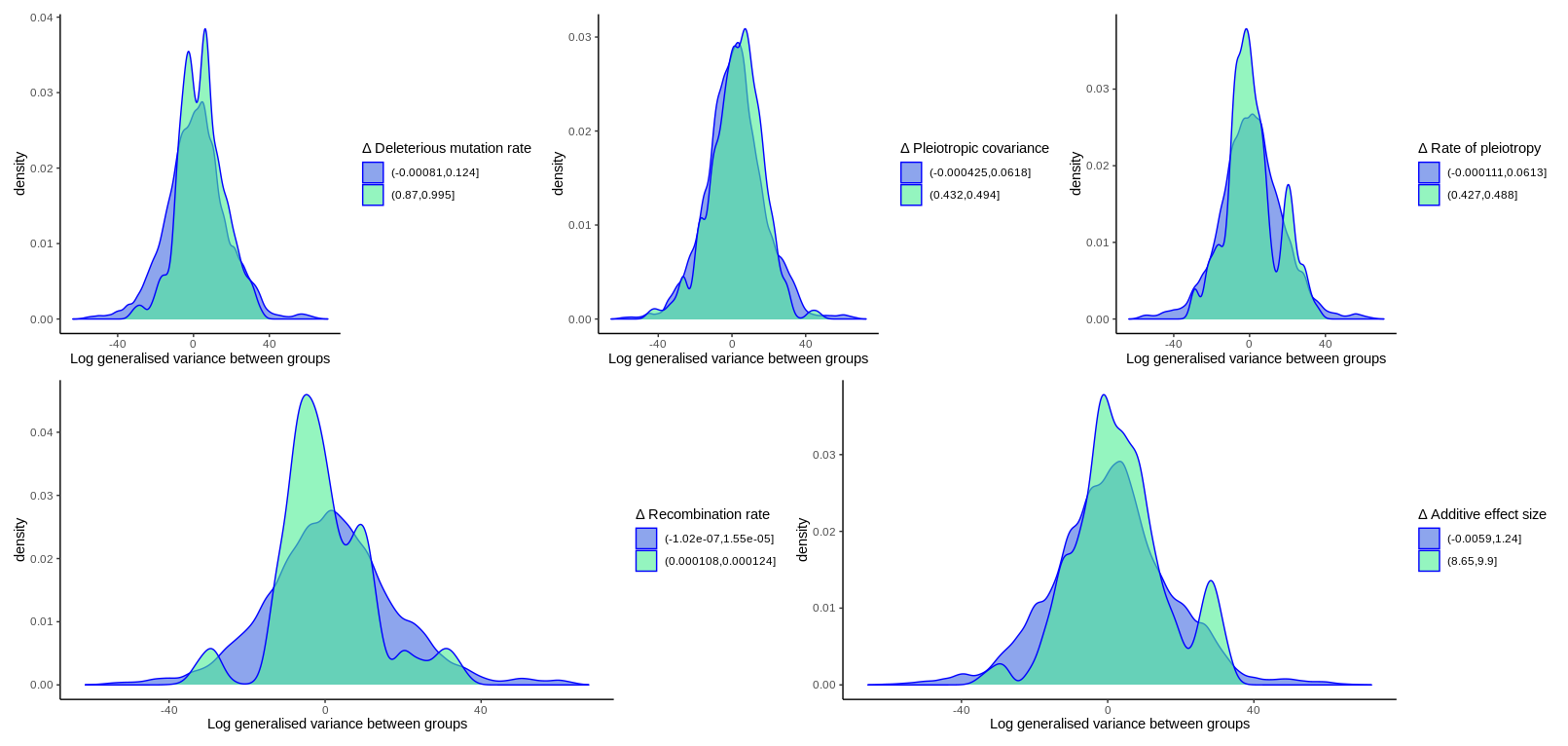
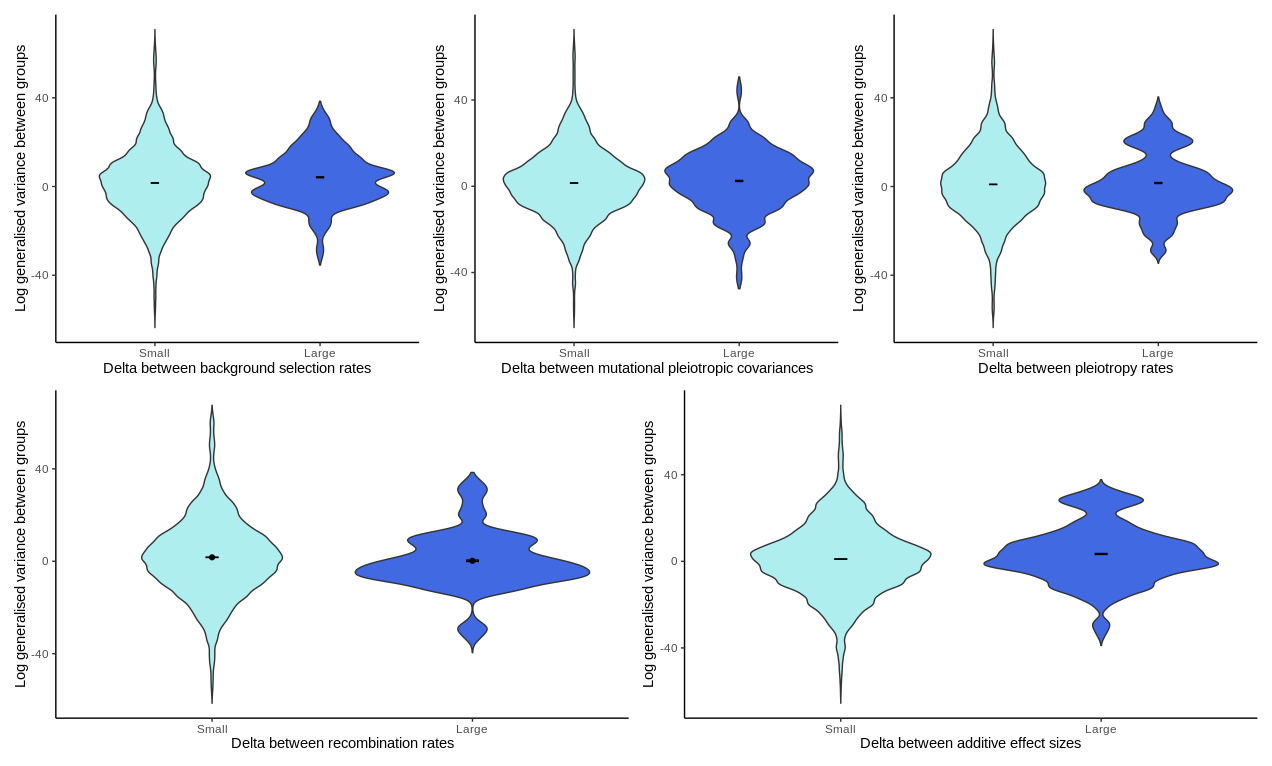
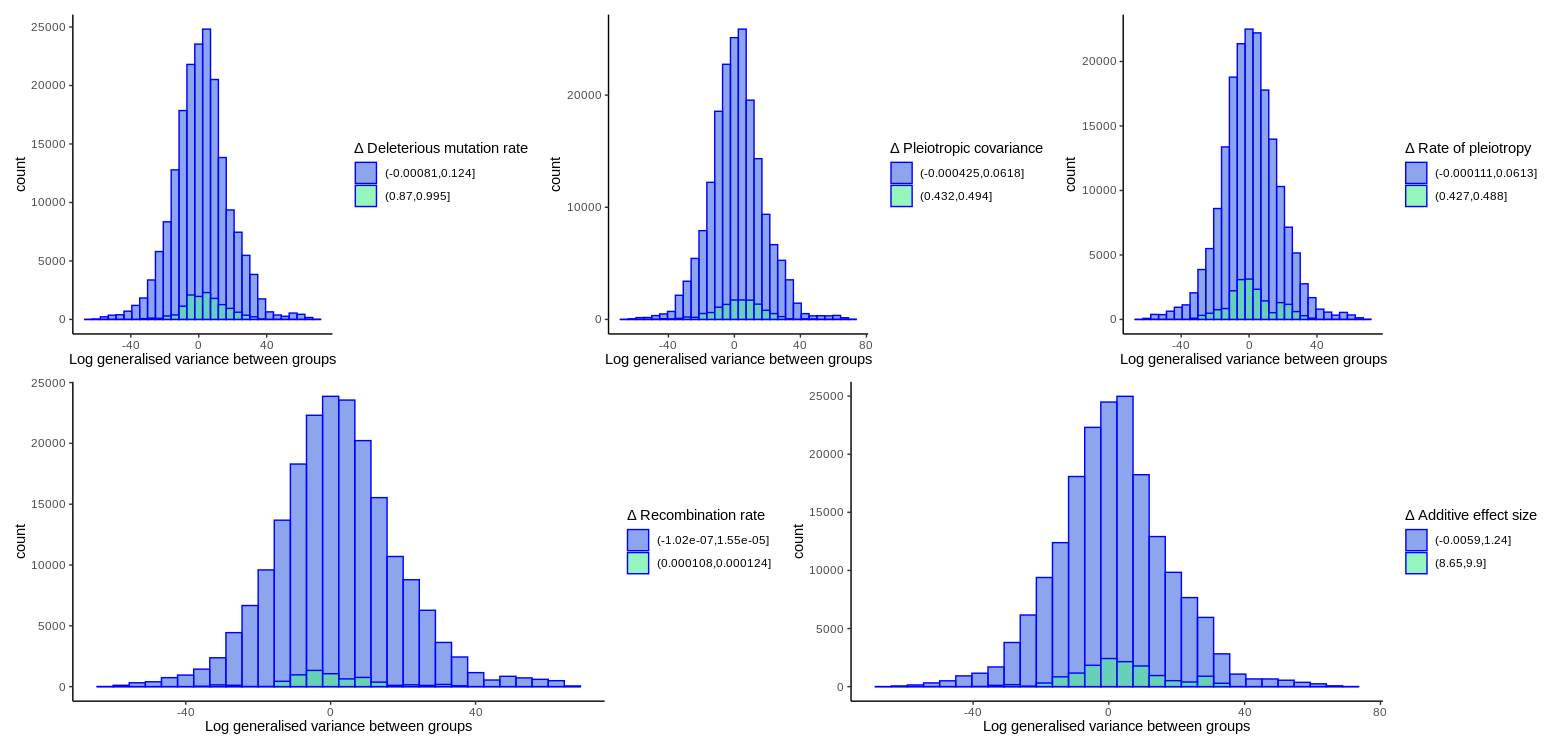


Figure 2 – Mean differences in log generalized variance between relative principal components analysis outcomes comparing similar models and distinctive models by deleterious mutation rate/background selection prevalence, mutational pleiotropic correlation, rate of pleiotropy, recombination rate, and additive effect size. All pairwise comparisons were significant.





One of these for Figure 3

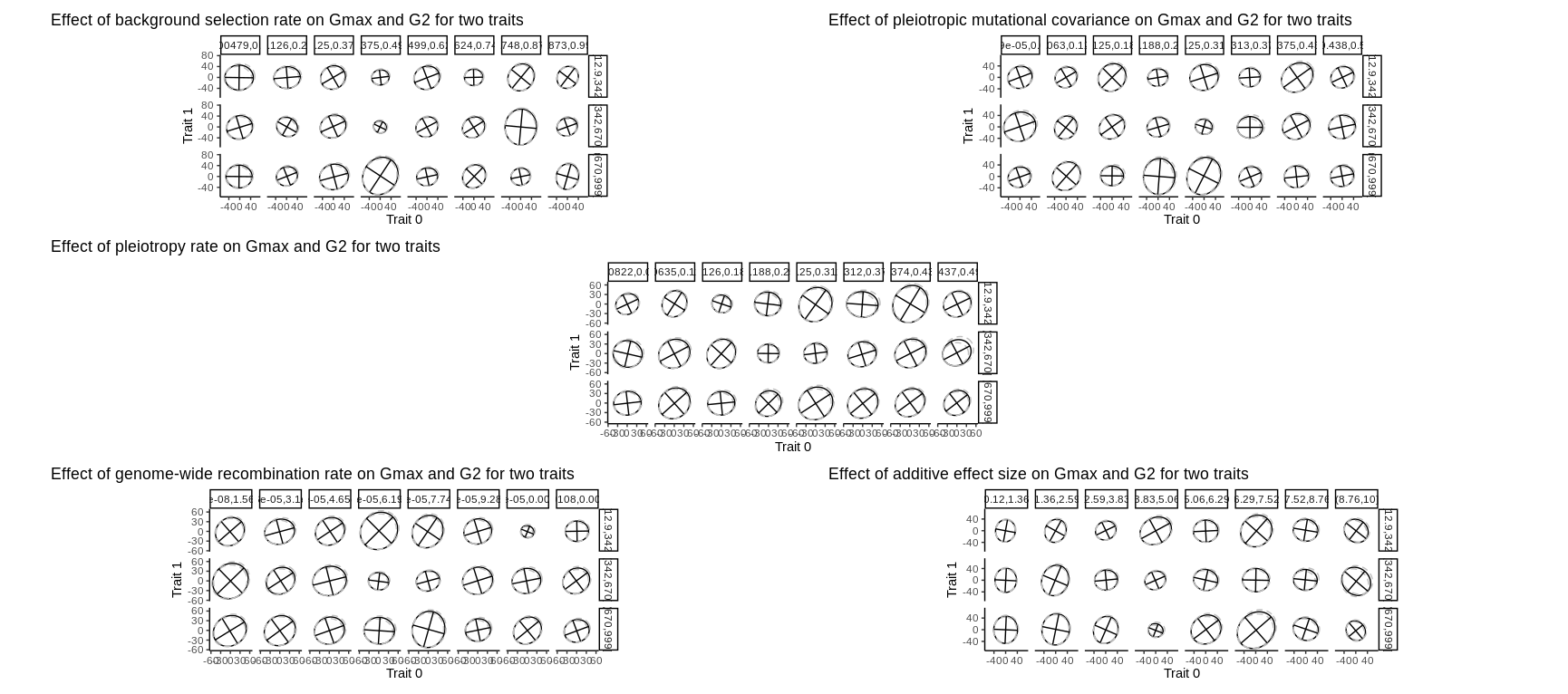


Figure 4 – **G** Ellipses for different parameters: left to right is increasing values of the given parameter, top to bottom is decreasing strength of selection.

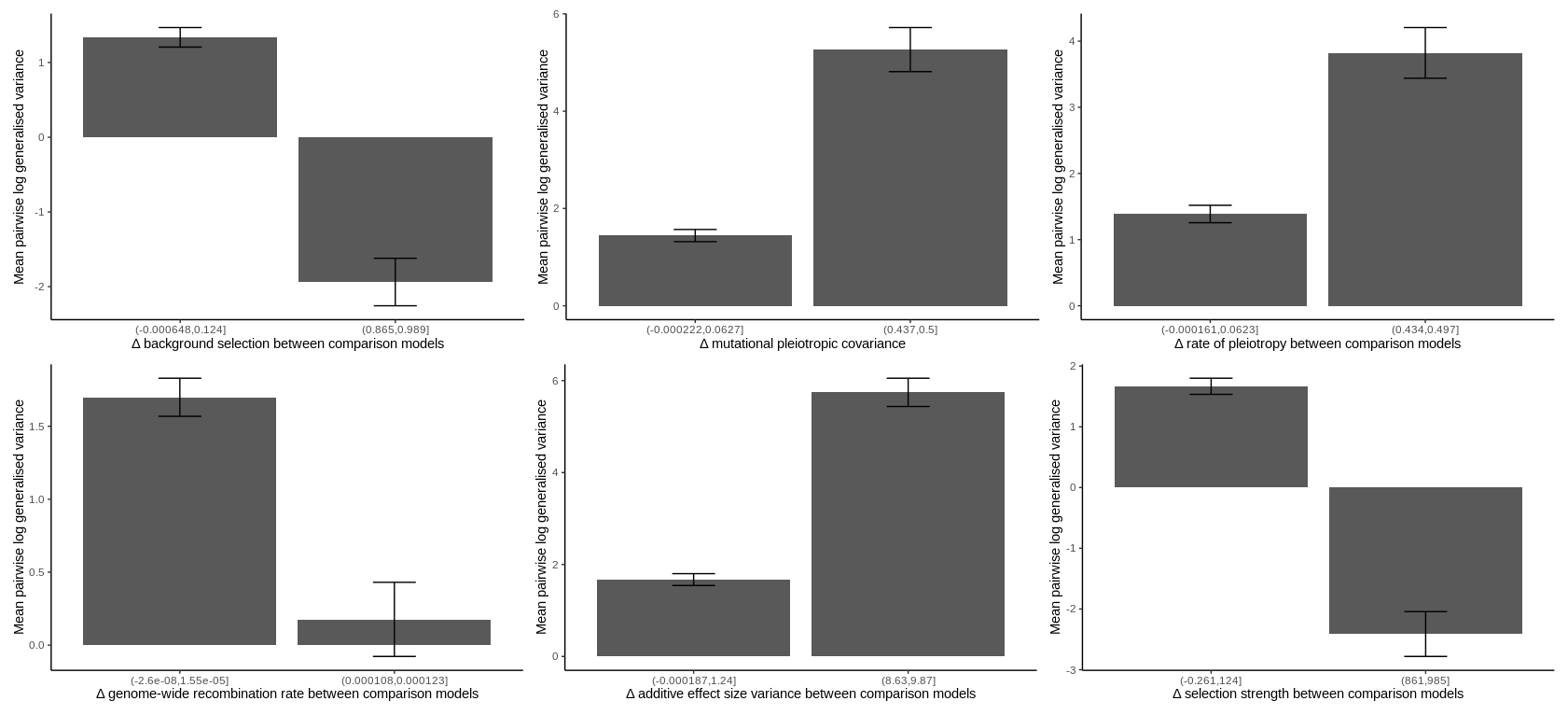
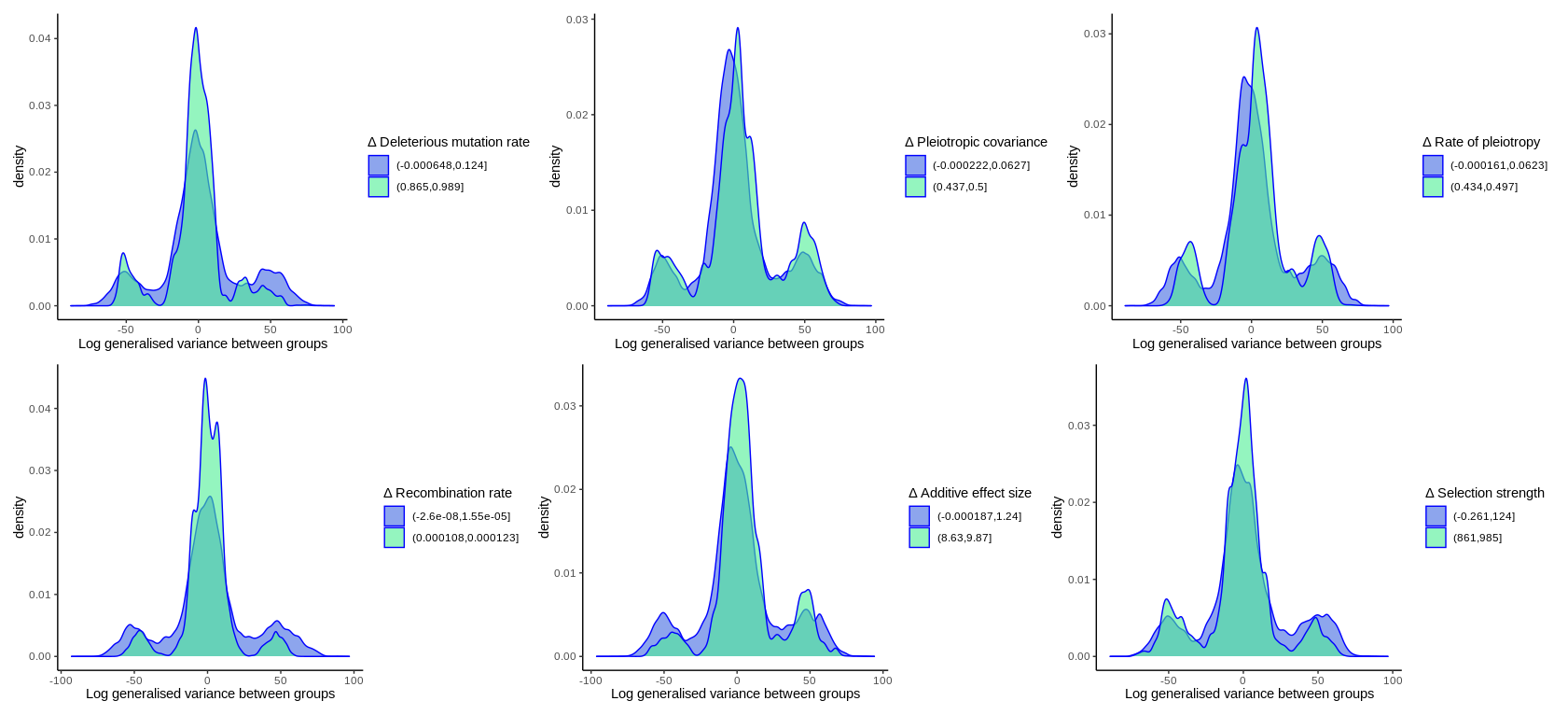
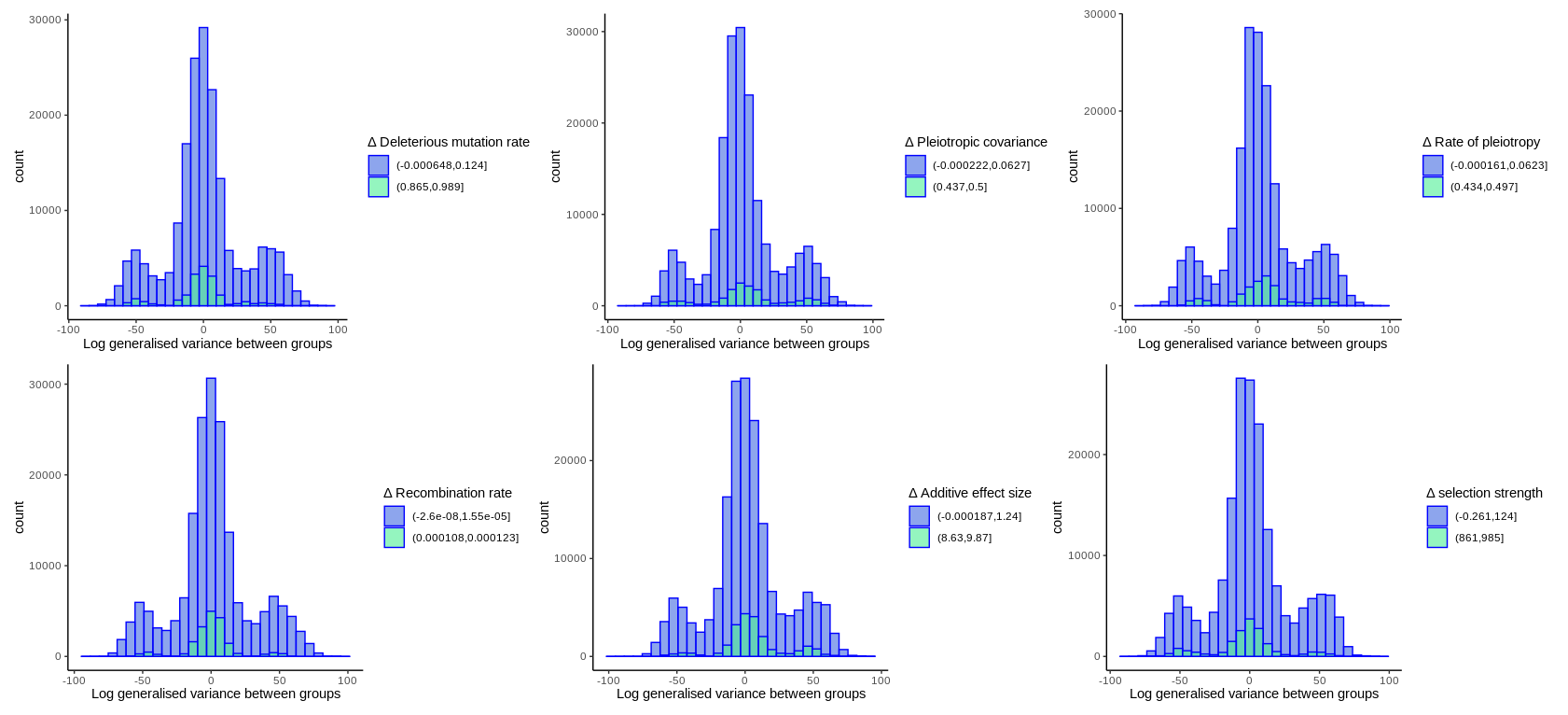
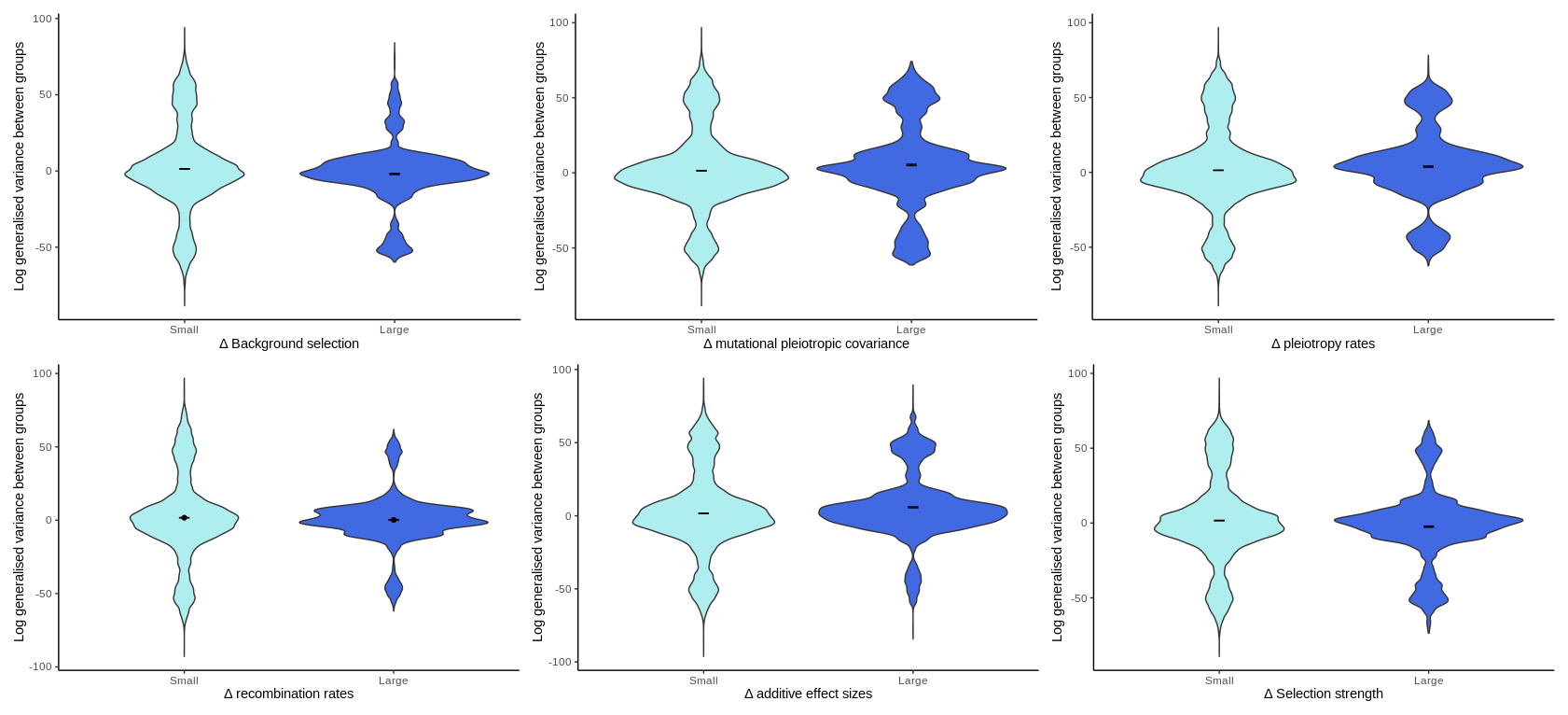


Figure 5 – Mean differences in log generalized variance among groups under selection





**One of these for figure 6**

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. |  |

Table 2: Test results for Kolmogorov-Smirnov tests between distributions of log generalized variance, the output of relative PCA comparing similar and distinct pairs of parameter models. All tests were two-sided.

|  |  |  |
| --- | --- | --- |
| Model parameter | D statistic | p-value |
| Deleterious mutation | 0.13168 | <0.0001 |
| Recombination rate | 0.11125 | <0.0001 |
| Pleiotropic correlation | 0.061734 | <0.0001 |
| Pleiotropy rate | 0.073615 | <0.0001 |
| Additive effect size | 0.1044 | <0.0001 |

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