The relative impact of evolving pleiotropy and mutational correlation on trait divergence.

Jobran Chebib and Frédéric Guillaume

Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. email: frederic.guillaume@ieu.uzh.ch

Abstract

Both pleiotropic connectivity and mutational correlations can restrict the divergence of traits under directional selection, but it is unknown which is more important in trait evolution. In order to address this question, we create a model that permits within-population variation in both pleiotropic connectivity and mutational correlation, and compare their relative importance to trait evolution. Specifically, we developed an individual-based, stochastic model where mutations can affect whether a locus affects a trait and the extent of mutational correlations in a population. We find that traits can diverge whether there is evolution in pleiotropic connectivity or mutational correlation but when both can evolve then evolution in pleiotropic connectivity is more likely to allow for divergence to occur. The most common genotype found in this case is characterized by having one locus that maintains connectivity to all traits and another that loses connectivity to the traits under stabilizing selection (subfunctionalization). This genotype is favoured because it allows the subfunctionalized locus to accumulate greater effect size alleles, contributing to increasingly divergent trait values in the traits under directional selection without changing the trait values of the other traits (genetic modularization). These results provide evidence that partial subfunctionalization of pleiotropic loci may be a common mechanism of trait divergence under regimes of corridor selection.

Keywords: Pleiotropy, Correlated Mutation, Genetic Architecture, Correlational Selection, Modularity, Mutation

Introduction

One of the central problems in evolutionary biology is understanding the processes through which new traits arise. One process that can lead to the creation of new traits is when existing traits become differentiated from one another because they are selected for a new purpose (Rueffler et al., 2012). There has long been evidence that this occurs through gene duplication followed by trait divergence (Muller, 1936; Ohno, 1970; Rastogi and Liberles, 2005). One example in vertebrates is the differentiation of forelimbs from hind limbs, where the same gene that was responsible for both fore and hind limb identity in development diverged (Graham and McGonnell, 1999; Minguillon et al., 2009; Petit et al., 2017). In this case, the paralogous genes *Tbx4/Tbx5* that encode transcription factors for fore/hindlimb identity likely evolved from the same ancestral gene, and their expression differentiated after duplication (Minguillon et al., 2009). Somehow during functional divergence, there was a break in the constraints of genetic architecture that determined the variational ability of the traits to respond to selection as independent modules (Wagner and Altenberg, 1996; Hansen, 2006).

Modular structure in phenotypic covariation is found in a wide range of organisms, including yeast, round worms and mice (Wang et al., 2010). The underlying genetic architectures producing this covariation between traits are beginning to become clearer, but are still uncertain. Constraints may stem from pleiotropic connections between loci and traits, where they may or may not create genetic covariation (Baatz and Wagner, 1997; Kenney-Hunt et al., 2008; Smith, 2016). The constraining effect of pleiotropy comes in two forms: a multi-trait genic effect or a multi-trait mutational effect (Stern, 2000). A multi-trait genic effect depends on how highly pleiotropic a gene is. For instance, a gene product (e.g. enzyme, transcription factor,

etc.) may affect more than one trait by having multiple substrates or binding sites. This may constrain the evolution of separate traits because the overall fitness effect of a mutation beneficial for one trait will have a larger deleterious effect proportional to the number of traits it affects (when those other traits are under stabilizing selection; Orr, 2000; Welch and Waxman, 2003). A multi-trait mutational effect is when a single mutation induces correlated changes in more than one trait. Of course, pleiotropic mutations are dependent on the gene being pleiotropic, but correlations in mutational effects create genetic correlations which can constrain trait divergence in addition to the constraints caused by pleiotropic genic effects (Lande, 1979; Arnold, 1992; Stern, 2000). We here separate the two effects of pleiotropy because a genic effect can constrain trait evolution even without creating genetic correlation among traits (Wagner, 1989; Baatz and Wagner, 1997). Yet, both types of pleiotropic effects can evolve as a result of directional selection to allow for the differentiation of traits, but it is not known which is most important in constraining or facilitating evolution of trait modularity (Chebib and Guillaume, 2017).

When there is genetic variation in the pleiotropic degree of genes or in the extent of correlation in mutational effects in a population, they can both evolve in response to selection (Jones et al., 2007; Pavlicev et al., 2011; Guillaume and Otto, 2012; Jones et al., 2014; Melo and Marroig, 2015). Previous models of evolution of pleiotropy looked at the effect of selection on genetic architecture evolution, and their predictions are all dependent on the details of selection (Melo et al., 2016). Pavlicev et al. (2011) developed a deterministic model of the evolution of pleiotropic gene effects under selection where polymorphic loci (rQTLs) could affect the strength of correlation between traits. Selection on all traits in the same direction favoured alleles that increased the correlations between traits. Whereas, when there was directional selection on one trait and stabilizing selection on another (called the corridor model of selection), alleles that lowered correlations between traits were favoured by

evolution. Melo and Marroig (2015) developed an individual-based, stochastic model where pleiotropic connections between 500 additive loci and ten traits were variable and mutable. They found similar results where directional selection on all traits in a module led to increased correlations between traits. Under the corridor model of selection, traits diverging from another became separate modules with fewer between-module pleiotropic connections and lower genetic correlations. But neither of these models explicitly allowed correlational mutations to evolve (e.g., mutational effects are completely correlated in Melo and Marroig, 2015). Another group of researchers allowed mutational correlation between two traits to evolve in simulation by allowing it to be determined by ten unlinked, additive loci (Jones et al., 2003, 2007). They found that patterns of mutational correlation evolved to match correlational selection but only investigated stabilizing selection without divergence between traits, and also did not allow for pleiotropic connections to evolve.

Both pleiotropic connections (genic effects) and mutational pleiotropy (mutational effects) affect the ability of traits to diverge from one another. Whether pleiotropic connectivity or mutational correlation is more important remains to be seen (Chebib and Guillaume, 2017). Here we attempt to answer this question using stochastic simulations of populations, where individuals can vary in both pleiotropic connections and mutational effects, and divergent selection on some traits but not others will lead to divergent trait evolution.

Methods

Simulation development

We modified the individual-based, forward-in-time, population genetics simulation software Nemo (v2.3.46) (Guillaume and Rougemont, 2006) to allow for the evolution of pleiotropic connectivity and mutational correlations at two quantitative loci affecting four traits. Mutations at the two QTL appeared at rate μ with allelic effects randomly drawn from a multivariate Normal distribution with constant mutational allelic variance $\alpha^2 = 0.1$ and

whose dimensions and covariance depended on individual-based variation in the pleiotropic connectivity of the QTL and in the loci coding for mutational correlations. This was done by implementing the capability for the pleiotropic connections between loci and traits in diploid individuals to be removed or added at a rate given by the pleiotropic mutation rate (μ_{pleio}), which determined whether a trait-specific allelic effect of one of the QTL was added to a trait value in that individual (Figure 1A). Each individual had a separate set of six quantitative loci, each affecting the mutational correlation between one pair of traits and which mutated at a rate given by the pleiotropic mutation rate (μ_{mutcor}) (Figure 1B). The two allelic values at those loci were averaged to give the covariance between the allelic effects of a pleiotropic mutation at the QTL affecting the traits. Therefore, whenever a mutation occurred at one of the two pleiotropic QTL, the mutational allelic effects were determined from the individual-specific connectivity matrix and mutational effects variance-covariance matrix $\mathbf{M}$ that gave the parameter values for the multivariate Normal distribution. Mutational effects were then added to the existing allelic values (continuum-of-alleles model; Crow and Kimura, 1964).

Experimental design

To understand the impact of directional selection on the structure of genetic architecture, simulations were run with a population of individuals that had two additive loci underlying four traits. The initial conditions were set to full pleiotropy (each locus affecting every trait) and strong mutational correlations between trait pairs ($\rho_\mu = 0.99$). This way, mutational effects in phenotypic space were highly constrained to fall along a single direction. The genotype-phenotype map was thus fully integrated, without modularity. Traits all began with a phenotypic value of 2 with equal value of 0.5 at each allele of the two causative QTL (loci are purely additive). Gaussian stabilizing selection was applied and determined the survival probability of juveniles, whose fitness was calculated as $w = \exp\left[-\frac{1}{2}\left((\mathbf{z}-\theta)^{\mathrm{T}} \cdot \mathbf{\Omega}^{-1} \cdot (\mathbf{z}-\theta)\right)\right]$,

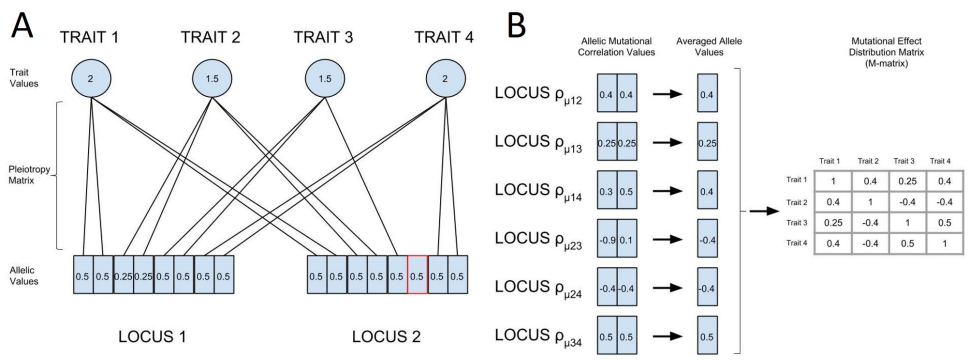

Figure 1: Pictorial representation of simulation model. A - Genetic architecture with an example of allelic values (genotype) of 2 loci, trait values (phenotype) of 4 traits, and the pleiotropic connectivity between them. Here, Trait 1 (and Trait 4) has a value of 2 determined by the sum of allelic values connected to it from Locus 1 (0.5 + 0.5) and Locus 2 (0.5 + 0.5). Traits 2 and 3 have the same value of 1.5 but for different reasons. For Trait 2, both alleles of Locus 1 have a value of 0.25 and both alleles of Locus 2 have a value of 0.5 (0.25+0.25+0.5+0.5=1.5), whereas for Trait 3 both alleles of Locus 1 are connected to it but only one allele of Locus 2 (0.5+0.5+0.5+0=1.5). B - An example of the creation of the mutation matrix (**M**-matrix) used for random sampling multivariate mutational effects with 6 loci representing each pairwise combination between 4 traits in an individual. Each locus has 2 alleles whose values represent correlations in mutational effects between traits that are averaged together and combined with the averages of the other mutational correlation loci into the off-diagonal elements of the **M**-matrix.

where $\mathbf{z}$ is the individual trait value vector, θ is the vector of local optimal trait values (all values initialized at 2), and $\mathbf{\Omega}$ is the selection variance-covariance matrix ($n \times n$, for n traits) describing the multivariate Gaussian selection surface. The $\mathbf{\Omega}$ matrix was set as a diagonal matrix with diagonal elements $\omega^2 = 5$ (strength of selection scales inversely with ω^2), and off-diagonal elements set for modular correlational selection with no correlation between modules ($\rho_{\omega 13} = \rho_{\omega 14} = \rho_{\omega 23} = \rho_{\omega 24} = 0$) and strong correlation within modules ($\rho_{\omega 12} = \rho_{\omega 34} = 0.9$). Divergent directional selection proceeded by maintaining static optimal trait values for traits 3 and 4 ($\theta_3 = \theta_4 = 2$) and increasing the optimal trait values for traits 1 and 2 by 0.001 per generation for 5000 generations, bringing the trait optima to $\theta_1 = \theta_2 = 7$ (corridor model of selection *sensu* Wagner, 1984; Bürger, 1986).

In order to compare the differential effects of evolving pleiotropic connections and evolving mutational correlations on trait divergence, nine different simulations were run with all combinations of three different rates of mutation in pleiotropic connections and mutational correlations (μ_{pleio} and μ_{mutcor} = 0, 0.001 or 0.01) representing no evolution, and mutation rates below, at and above the allelic mutation rate ($\mu = 0.001$), respectively. Simulations were also run with initial mutational correlations between all pairs set to 0 ($\rho_\mu = 0$) to compare highly constrained genetic architecture to ones with no constraints in the direction of mutational effects.

Unless otherwise specified, each simulation was run with 500 initially monomorphic (variation is introduced through mutations) individuals for 5000 generations of divergent, directional selection on traits 1 and 2 (followed by 5000 generations of stabilizing selection) in order to observe general patterns of average trait value divergence, population fitness, genetic correlation, pleiotropic degree and mutational correlation. In the case of pleiotropic degree, the two loci affecting trait values were sorted into a high and low pleiotropic degree locus for each individual before averaging over populations or replicates so that differential effects of the two loci were not averaged out in the final analysis. Individuals were hermaphrodites mating at random within a population, with non-overlapping generations. Statistics were averaged over 50 replicate simulations of each particular set of parameter values.

Results

Trait divergence and genetic modularity under genetic constraints

In the absence of genetic architecture evolution ($\mu_{pleio} = \mu_{mutcor} = 0$), trait modules are still capable of divergence, but do not follow trait optima closely since traits 3 and 4 get pulled away from their optima as traits 1 and 2 increase to follow theirs (Figure 2). With the introduction of variation in genetic architecture through mutation (μ_{pleio}, $\mu_{mutcor} > 0$), average trait

values follow their optima more closely and the capability of trait divergence increases as mutation rates in genetic architecture increases, which leads to higher average population fitness values by generation 5000 (Figure 3). Also by generation 5000, simulations with higher pleiotropy mutation rates ($\mu_{pleio} \geq 0.001$ or $\mu_{mutcor} = 0.01$) have distinctly modular genetic correlation structures with stronger correlations between traits 1 and 2 than between traits 3 and 4 (Figure 4). But at the highest pleiotropy mutation rate ($\mu_{pleio} = 0.01$) the genetic integration of trait 3 with 4 (and even trait 1 with 2) is no longer as strong. An increase in pleiotropic connection evolution has a greater effect on trait divergence and genetic modularization than the same increase in mutational correlation evolution, which is evident when either μ_{pleio} or μ_{mutcor} is the same as the allelic mutation rate ($\mu = 0.001$). Even when the mutational correlation mutation rate is at the highest tested ($\mu_{mutcor} = 0.01$), an increase in the pleiotropy mutation rate still improves the ability for traits to diverge, which can be seen in the decrease in variance over replicate simulations (Figure 2).

Effects of pleiotropic connectivity and mutational correlation evolution on rate and extent of divergent evolution

When evolution of pleiotropic connections is possible ($\mu_{pleio} > 0$), the most common allele in almost all cases is one that maintains connections to traits 1 and 2, but has lost connections to traits 3 and 4 after two mutational events. This allele is found in locus 1 or 2 at a frequency of 0.873 averaged over the populations of all simulations where evolution of pleiotropic connections is possible. The allele goes to fixation or near fixation in one locus where its pleiotropic degree decreases from four to two, and this happens more rapidly as μ_{pleio} increases (Figure 5). The decrease in pleiotropic degree resulting from the increase in frequency of this allele coincides with the modularization of genetic correlations, the divergence of traits and the increase in fitness. The proportion of times in which this particular allele becomes common in locus 1 or in locus 2 is approximately equal over all simulations (0.491 and

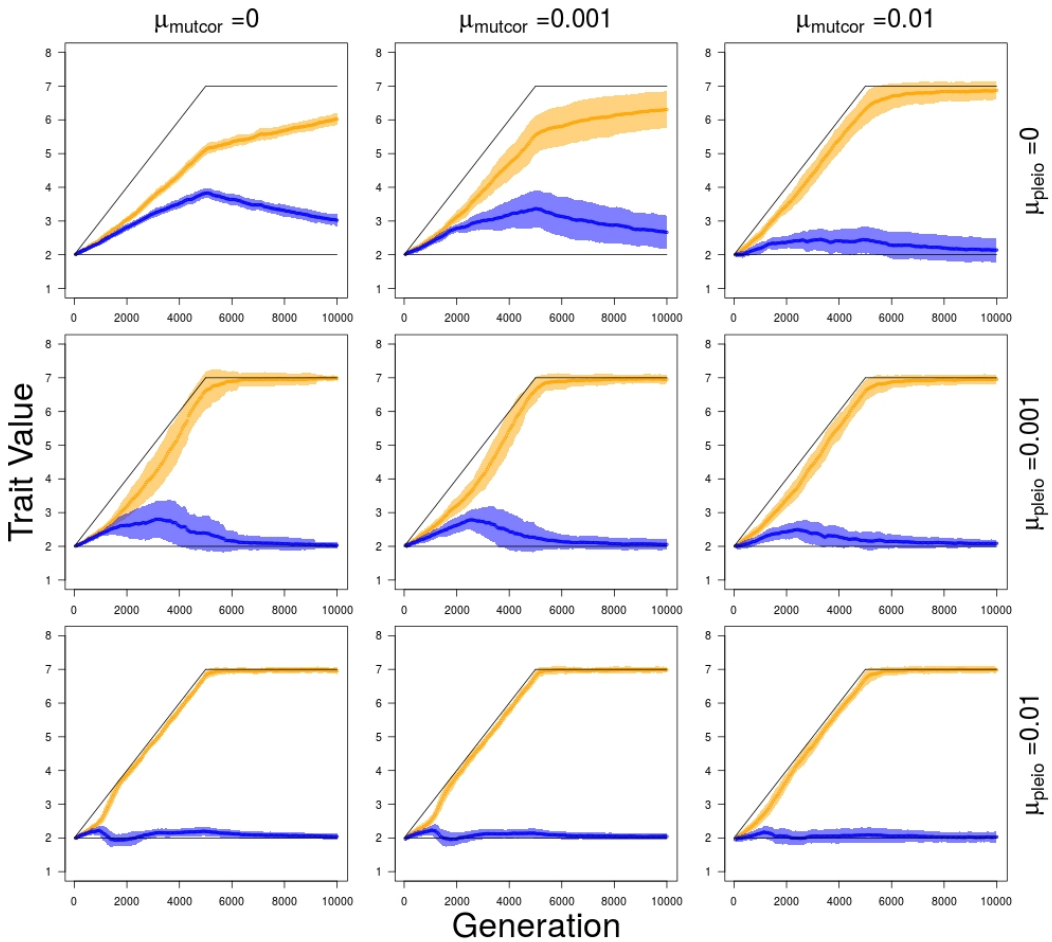

Figure 2: Trait value divergence over 5,000 generations of directional selection on traits 1 and 2 (Module 1) followed by 5,000 generations of stabilizing selection for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). Orange – average values of traits 1 and 2; Blue – average values of traits 3 and 4; Black – trait value optima for modules 1 and 2. Shaded regions show standard errors of the mean for 50 replicate simulations.

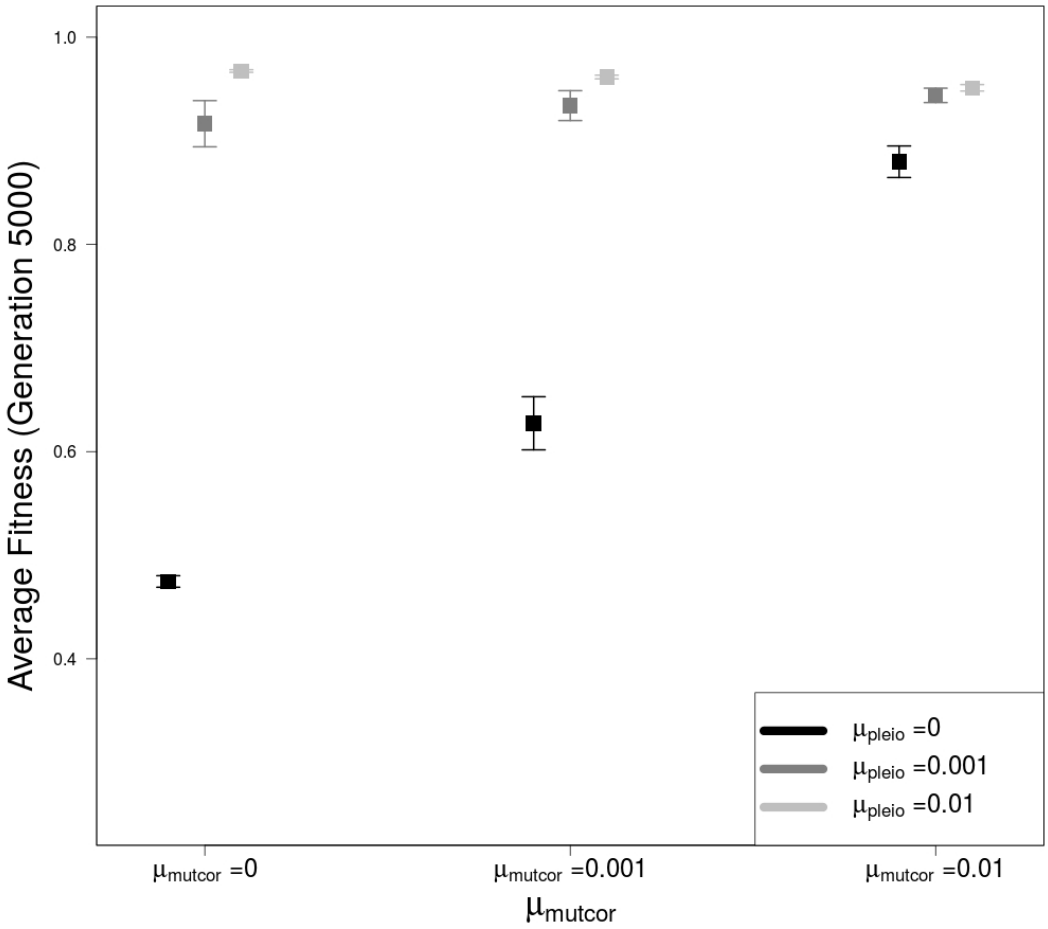

Figure 3: Average population fitness after 5,000 generations of directional selection on traits 1 and 2 (Module 1) for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). All error bars represent standard errors of the mean for 50 replicate simulations.

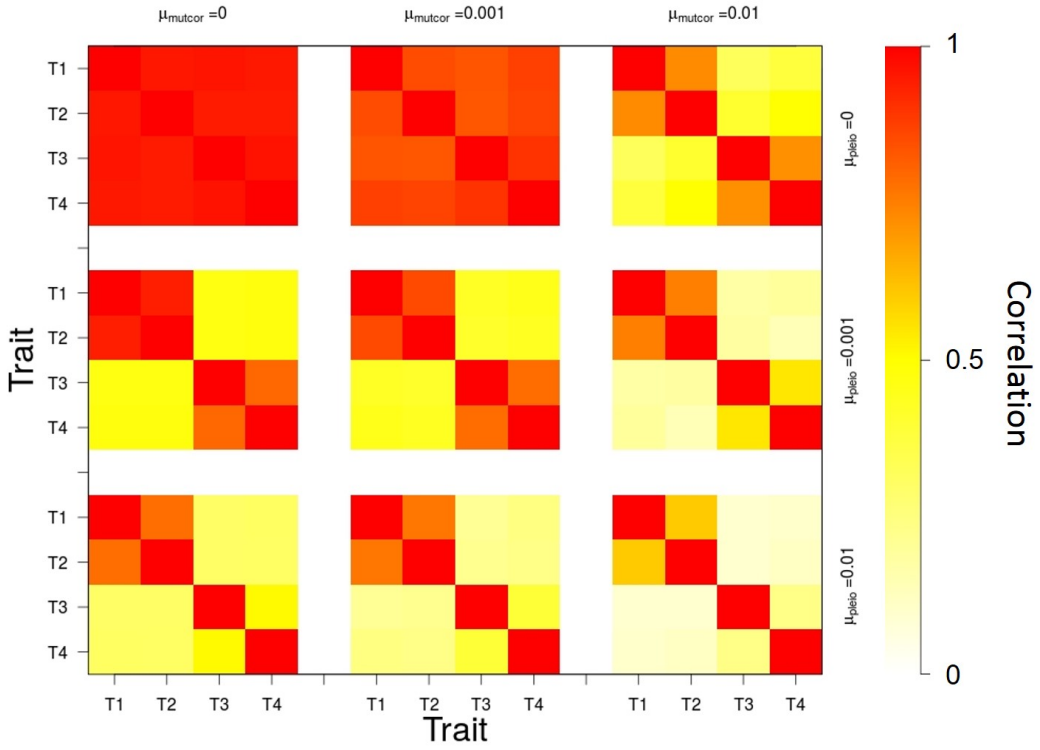

Figure 4: Genetic correlations between traits after 5,000 generations of directional selection on traits 1 and 2 (Module 1) for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). Red – higher genetic correlation. White – no genetic correlation

0.509, respectively, over 300 simulations) and is never observed in both loci in any one individual.

When the mutation rate for pleiotropic connections (μ_{pleio}) is zero, mutational correlation evolution can still lead to trait divergence but this takes longer, does not diverge as fully, and therefore leads to lower population mean fitness. Evolution of the mutational correlation occurs by a general decrease in all mutational correlations between traits at a rate determined by the mutation rate of mutational correlations (Figure 6). When the mutation rate at the mutational correlation loci is higher than the pleiotropic mutation rate then genotypic patterns do emerge where one locus disconnected from trait 3 combines with lower mutational correlations between traits 1 and 4 or 2 and 4, *or* a locus disconnected to trait 4 combines with lower mutational correlations between traits 1 and 3 or 2 and 3 (at frequencies of 0.16 and 0.10 over 50 replicates, respectively). But even in the case with a higher mutation rate for mutational correlation than the pleiotropic mutation rate, full subfunctionalization (one locus loses connections to traits 3 and 4) is a common outcome after 5,000 generations occurring at a frequency of 0.18 over 50 replicates.

Effect of mutational correlation initial conditions set to zero ($\rho_\mu = 0$ versus $\rho_\mu = 0.99$)

In simulations where all mutational correlations are initialized at zero, there is little to no constraint on trait divergence despite full pleiotropic connectivity. This can be observed in trait values that follow their optima closely, leading to little reduction in fitness as optima for traits 1 and 2 diverge from traits 3 and 4, with little evolution in mutational correlations and pleiotropic degree during divergent selection (Figure 7). There are still patterns of genetic architecture evolution as alleles with lowered pleiotropic degree still emerge in the populations, but fixation is not common nor are any allelic patterns of mutational correlations.

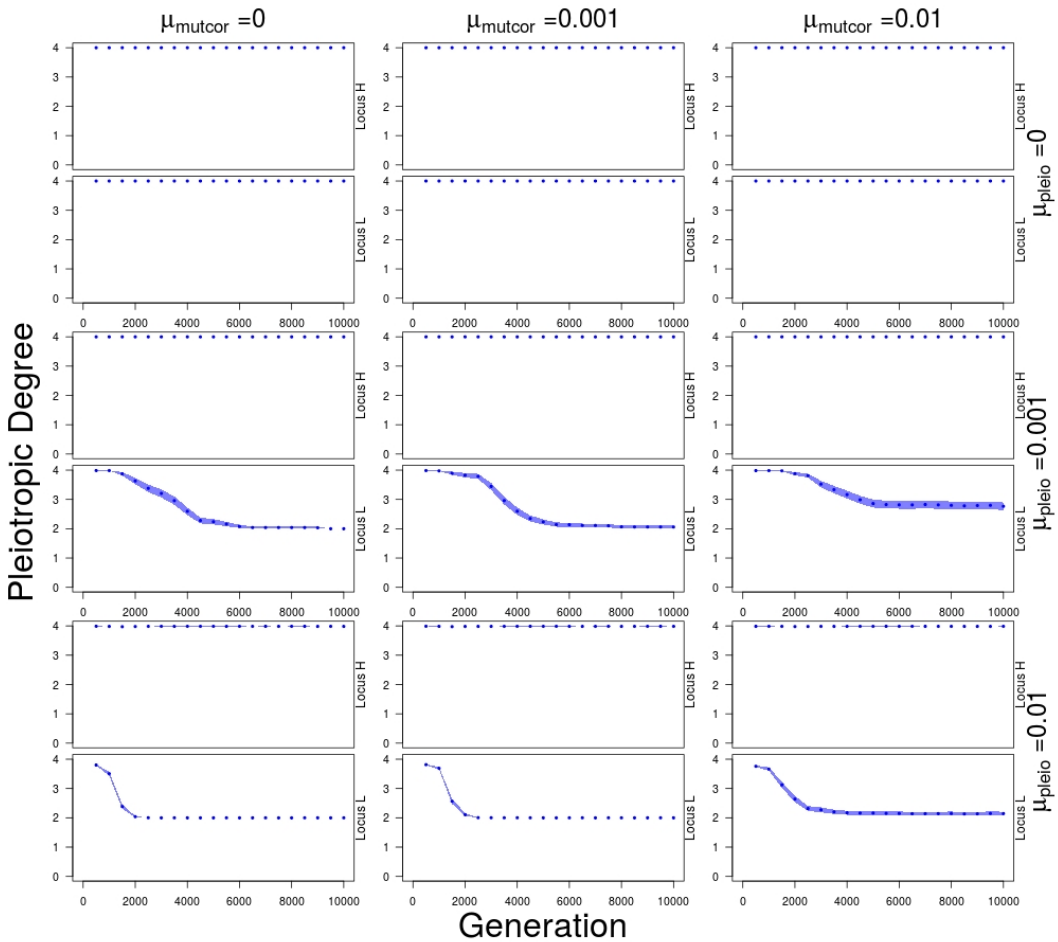

Figure 5: Average number of traits connected to each locus over 5,000 generations of directional selection on traits 1 and 2 (Module 1) followed by 5,000 generations of stabilizing selection for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). Loci are sorted so that locus with higher pleiotropic degree (Locus H) is always shown above and lower pleiotropic degree (Locus L) shown below. Shaded regions show standard errors of the mean for 50 replicate simulations.

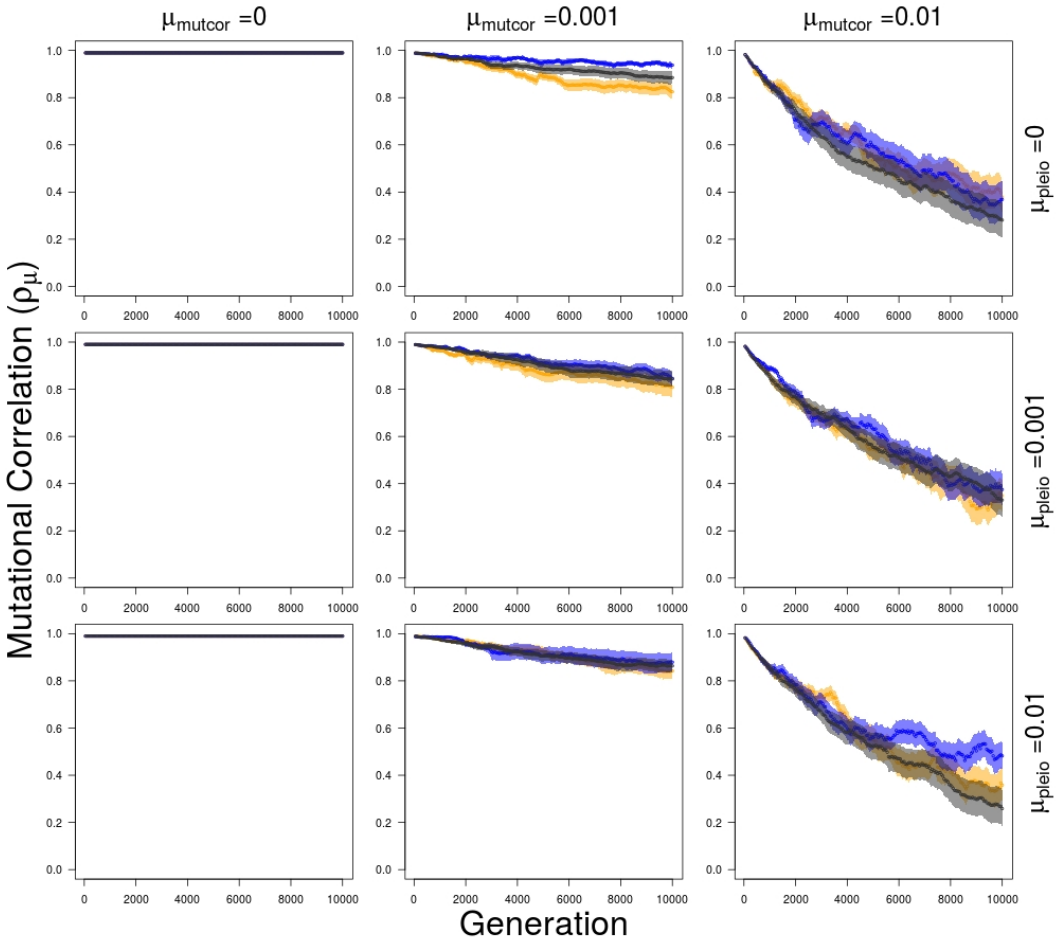

Figure 6: Average within and between module mutational correlation over 5,000 generations of directional selection on traits 1 and 2 (Module 1) followed by 5,000 generations of stabilizing selection for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). Orange – mutational correlation between traits 1 and 2 (within Module 1); Blue – mutational correlation between traits 3 and 4 (within Module 2); Black – average mutational correlations between traits 1 and 3, 1 and 4, 2 and 3, and 2 and 4 (between Module 1 and 2). Shaded regions show standard errors of the mean for 50 replicate simulations.

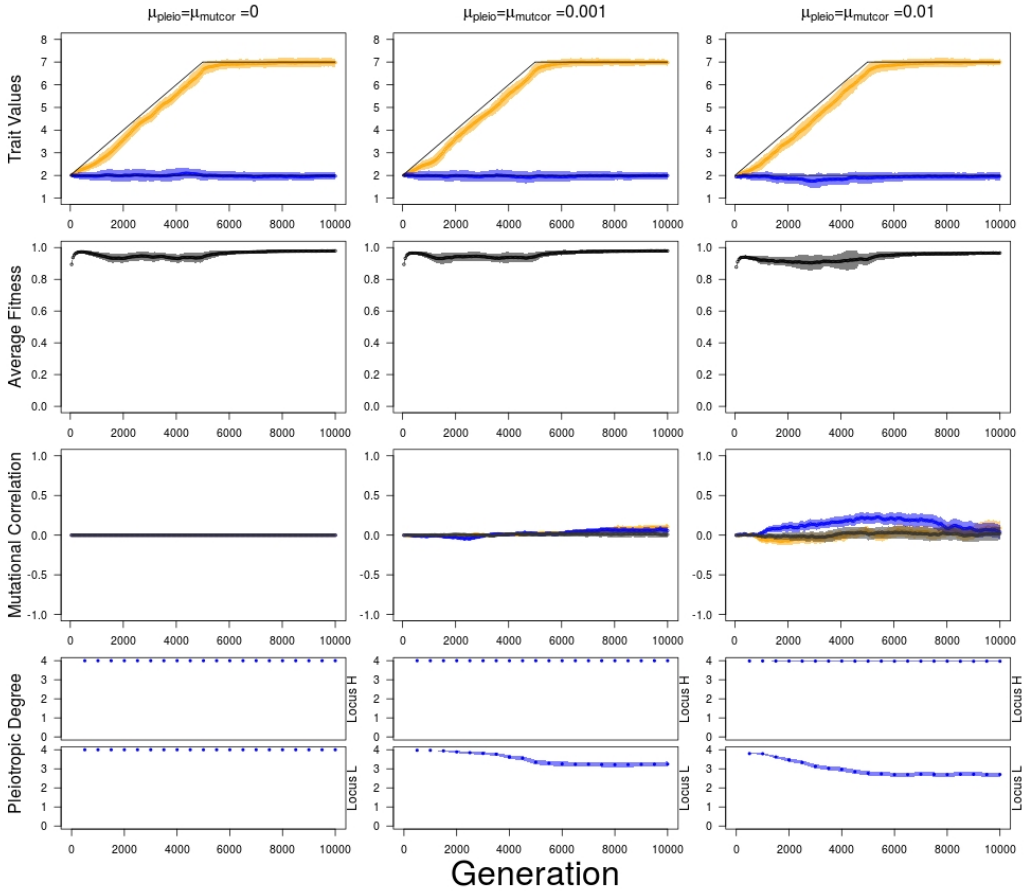

Figure 7: Average trait value, fitness, mutational correlation and pleiotropic degree, when all ρ_μ are initialized to 0, over 5,000 generations of directional selection on traits 1 and 2 (Module 1) followed by 5,000 generations of stabilizing selection for different combinations of mutation rate in pleiotropic connections (μ_{pleio}) and mutational correlations (μ_{mutcor}). For pleiotropic degree, loci are sorted so that locus with higher pleiotropic degree (Locus H) is always shown above and lower pleiotropic degree (Locus L) shown below. Orange – trait 1 and 2 values or mutational correlation between traits 1 and 2 (Module 1); Blue – trait 3 and 4 values or mutational correlation between traits 3 and 4 (Module 2); Black – average mutational correlations between traits 1 and 3, 1 and 4, 2 and 3, and 2 and 4 (between Modules 1 and 2). Shaded regions show standard errors of the mean for 50 replicate simulations.

Discussion

Pleiotropy and mutational correlation can lead to trait divergence

Previous models of genetic architecture evolution have shown that evolution in pleiotropy and mutational correlation can influence genetic correlation between traits and therefore responses to selection, but as far as we are aware this is the first time both have been allowed to evolve in the same model. When genetic architectures are highly constraining to the divergent evolution of some traits from others, then evolution of the structure of the genotype-phenotype map can clearly facilitate the rate and extent of trait divergence. Although genetic architecture may evolve through changes in pleiotropic connectivity between genes and traits, and in the mutational correlations between traits, the former leads to a greater release of genetic constraints and faster adaptation. A qualitative distinction exists between these two types of genetic constraints for two reasons. First, genetic constraints based on mutational correlation distributions are more difficult targets of selection compared to pleiotropic connections because mutations on modifiers of genetic correlations do not affect the trait phenotypes directly, whereas a single allele that differs in its pleiotropic connectivity does. Second, mutational correlations are dependent on pleiotropic connections to be effectual on traits, whereas the latter can affect the rate of adaptation regardless of mutational correlation (Baatz and Wagner, 1997; Chebib and Guillaume, 2017).

The results of this study corroborate results from previous models of pleiotropic evolution. We observe that divergent selection in the form of the corridor model leads to modular genetic architecture with greater genetic correlations between traits *within* modules and lower correlations *between* modules. This was also the case in both Melo and Marroig (2015) and Pavlicev et al. (2011) under the corridor model. Unfortunately, it is unclear whether patterns of partial modular pleiotropy that were responsible for the emergence of modularity in our study were also observed in these studies

because they did not report the most common resulting genotypes after corridor selection. Melo and Marroig (2015) did however vary the mutation rate in pleiotropic connections (while keeping allelic mutation rate the same) and found that when μ_{pleio} was 10 times greater than μ, there were higher within and between module correlation compared to when μ_{pleio} and μ were the same. Though our results corroborate this relationship as well, we cannot deduce the state of the pleiotropic connections that led to those results in their simulations. Their study also did not include evolution in mutational correlations so it is not possible to do a comparison on the relative effects of mutational correlation and pleiotropic connectivity on patterns of modularity. As previously mentioned, Pavlicev et al. (2011) had a deterministic model with rQTL that affected the correlations between traits directly instead of affecting the genotype-phenotype connections, making it difficult to compare patterns of partial modular pleiotropy. Jones et al. (2007) found "extreme" variation among replicates in the average mutational correlation observed when ρ_μ was capable of evolving, similar to what was observed in our study (as well as when simulations were run with the same parameter values as the Jones et al. (2007) study; Supplemental Figure S1). This variation the evolution of mutational correlation is likely due to an unstable equilibrium in the adaptive landscape in which highly positive or negative mutational correlations have a selective advantage over mutational correlations closer to zero (Lande (1980); Zhang and Hill (2002); Jones et al. (2007); Supplemental Figure S2).

Patterns of pleiotropy

What explains the emergence of one dominant genotype that has one locus losing its connections to traits 3 and 4, and the other locus maintaining full pleiotropy? When mutational correlations are strong, modularization should arise so that mutational effects can increase traits 1 and 2 values without also increasing traits 3 and 4, (especially when stabilizing selection is strong compared to directional selection). If stabilizing selection had been weaker

and/or directional selection been much stronger, then more loci affecting the traits would have increased the proportion of advantageous mutations allowing for divergence (Hansen, 2003). For the same reason, we don't observe complete modularization with one locus only connected to traits 1 and 2, and the other only connected to traits 3 and 4. With both loci contributing to traits 1 and 2, there is more mutational input to increase their values, giving support to the idea that intermediate levels of integration will maximize evolvability when pleiotropic effects are all positive (Hansen, 2003). Also, since we were interested in the evolution of genetic architecture with trait divergence after gene duplication, we started our simulations with a highly constrained, monomorphic population. This makes evolution in our model dependent on de-novo mutations and as traits diverged, the negative effects of pleiotropy on traits under stabilizing selection increased, leading to modularization in the genetic architecture. But if we had simulated genetic architectures where the allelic mutation rate (μ) was high enough and selection acted on many loci with small effects, pleiotropy would not have been as constraining, and integrated genetic architectures (loci affecting all traits) would have been more evolvable (as shown in the analytical model of Pavlicev and Hansen (2011)). This also would have been true if standing genetic variation had already existed in pleiotropic connections and mutational correlations for duplicated loci in a population prior to divergent selection. We could imagine that many possible combinations of pleiotropic connection and mutational correlation alleles that allow for increased variation and reduced covariation between traits could also exist. In that scenario, modularization may not be associated with trait divergence.

The results we obtain in this study are also related to work done on the evolutionary fate of duplicated, pleiotropic genes (Ohno, 1970; Hahn, 2009; Innan and Kondrashov, 2010; Guillaume and Otto, 2012). Previous models describe the conditions under which both genes remained fully pleiotropic, which is expected to be favorable when there is selection for increased dosage

as we had for traits 1 and 2 (Ohno, 1970). There is some empirical evidence of this in ribosomal RNA, histone genes, as well as amylase genes in humans with high starch diets (Zhang, 2003; Perry et al., 2007; Qian et al., 2010). Other models describe when one or both genes lose their connection to some traits, known as subfunctionalization, if there is a relaxation of selection after duplication (Force et al., 1999; Lynch and Force, 2000). Empirical evidence for subfunctionalization exists for vertebrate limb evolution, as discussed in the introduction, as well as pathway specialization in plants (Bomblies and Doebley, 2006; Des Marais and Rausher, 2008). Compared to models with selection for increased dosage, our model has selection only for higher values in traits 1 and 2, whereas selection for increased values in all four traits is expected to maintain all pleiotropic connections. The difference compared to neutral models where subfunctionalization is the result is that in our model there is no relaxation of selection due to duplication and redundancy. In that case, Guillaume and Otto (2012) showed that the maintenance of pleiotropy in one gene and subfunctionalization in the other (the most common outcome in our simulations) is predicted when there is asymmetry in either the trait contributions to fitness or in the expression levels of the genes. The gene with higher expression was predicted to remain fully pleiotropic, with loss of pleiotropy in the second, less expressed gene. Our results fit very well with that later outcome, although the conditions were different. In Guillaume and Otto (2012), a fitness trade-off emerged from the competitive allocation of the gene product (amount of protein produced) between two traits under positive selection (i.e., increased allocation to one trait reduced allocation to the other trait). The fitness trade-off in our model arose from the corridor model of selection whereby increased additive contributions to traits 1 and 2 via fully pleiotropic mutations with correlated allelic values trade-off negatively with traits 3 and 4 under stabilizing selection. The trade-off is quickly attenuated when the correlation between the additive effects of the mutations decreases. Mutation of the pleiotropic degree of the causative loci was nevertheless more

efficient in breaking the constraint. It is also a more plausible mechanism since mutations in transcription binding sites may cause such drastic change in pleiotropic connections.

Empirical evidence for mutational correlation and pleiotropy

The pleiotropic mutational effects in our model abstract out the types of molecular level changes that may lead to changes in genetic correlations between traits. Some examples of variation in pleiotropic connections come from empirical studies on transcriptional regulation. For example, expression of the *Tbx4* gene (described earlier) is required not only for hindlimb development but is also expressed in genital development (Chapman et al., 1996). Although the upstream enhancer of *Tbx4*, hindlimb enhancer B (or HLEB), is functional in both hindlimb and genital development in both mice and lizards, HLEB appears to have lost its hindlimb enhancer function in snakes due to mutations in one of the enhancer's binding regions (Infante et al., 2015). A more recent example comes from two species of Drosophila the diverged only 500,000 years ago. *D. yakuba* has both hypandrial and sex comb bristles whereas *D. santomea* has only sex comb bristles (Rice and Rebeiz, 2019). Quantitative trait mapping crosses between the species and with *D. melanogaster* revealed that a single nucleotide change in a regulatory enhancer of the *scute* gene, which promotes bristle development, was responsible for *D. santomea* losing its hypandrial bristles and increasing its sex comb bristle number (Nagy et al., 2018). These examples provide evidence that mutations in DNA binding sites can affect a gene's pleiotropic degree, allowing for evolution of trait modularity.

Correlated mutational effects, on the other hand, may arise from mutations that cause correlated effects in more than one of a gene's molecular functions or from mutations causing correlated effects in a gene product's multiple processes, but empirical data is still needed to discover the source of mutational correlation (Hodgkin, 1998; Wagner and Zhang, 2011). Even if the specific molecular mechanism that is the cause of correlation is not known,

it is still possible to estimate the genomic **M**-matrix which describes the combined pattern of (co)variation arising from mutations in all loci that affect the traits of interest. Mutation accumulation experiments in *D. melanogaster* (Houle and Fierst, 2013) or *C. elegans* (Estes et al., 2005) provide examples of such genomic **M**-matrix estimates and show the existence of strong mutational correlation among morphological and life-history traits. Additionally, mutational correlations in *C. elegans* seem to correspond to phenotypic correlations among traits after removing environmental correlations and suggest that pleiotropy is somewhat restricted within traits of related function (Estes et al., 2005).

It is also possible to discover evidence of modular pleiotropy from genome-wide studies using gene knock-out/-down experiments as was performed in yeast (Dudley et al., 2005; Güldener et al., 2005; Ohya et al., 2005), *C. elegans* (Sönnichsen et al., 2005), and the house mouse (Bult et al., 2008), which have shown that whole-gene pleiotropy is variable (does not affect all traits) and often modular (Wang et al., 2010; Wagner and Zhang, 2011). QTL studies further show variable pleiotropy in *D. melanogaster* (Mezey et al., 2005), threespine stickleback (Albert et al., 2008), the house mouse (Cheverud et al., 1997; Kenney-Hunt et al., 2008; Miller et al., 2014), and *A. thaliana* (Juenger et al., 2005), among others (Porto et al., 2016).

One empirical study manages to link mutational correlation with modular variation of pleiotropy by measuring both the genomic **M**-matrices and the pleiotropic degree of main and epistatic effects of mutations affecting the replicative capacity (fitness) of HIV-1 in different drug environments (Polster et al., 2016). In doing so, they discovered that epistasis can affect the pleiotropic degree of single mutations producing modular GP maps and that epistatic-pleiotropic effect modules matched modules of fitness co-variation among drugs. These results suggest that epistasis may be fundamental in shaping the GP map itself, which may allow organisms to enhance their evolvability in the face of selection (Pavlicev et al., 2008, 2011; Pavlicev and

Cheverud, 2015).

Conclusion

Both pleiotropic connections and mutational correlations can constrain the divergence of traits under directional selection, but when both can evolve, trait divergence occurs because pleiotropic connections are broken between loci and traits under stabilizing selection. The evolution of pleiotropic connectivity is favoured because it is an easier target of selection than a distribution of mutational effects. The most commonly observed genotype thus includes one locus that maintains connectivity to both traits under directional selection and both traits under stabilizing selection, and the other locus losing its connection to the traits under stabilizing selection (subfunctionalization). The subfunctionalization of one locus allows it to contribute to increasingly divergent trait values in the traits under directional selection without changing the trait values of the other traits, which leads to separate genetic modules. These results indicate that partial subfunctionalization is sufficient to allow genetic modularization and the divergence of traits with little to no loss of average fitness.

Acknowledgments

This manuscript benefited from the comments and constructive criticism provided by Thomas F. Hansen. J.C. and F.G. were supported by the Swiss National Science Foundation, grant PP00P3_144846 to F.G.

Author Contributions

J.C. performed software modification for model implementation and acquisition of data, as well as drafting of manuscript. J.C. and F.G. performed study conception and design, analysis and interpretation of data, and critical revision of manuscript.

Data Archival

The data for this study is available online through Dryad Digital Repository at https://datadryad.org/search?utf8=%E2%9C%93&q=chebib

Conflict of interest disclosure

The authors of this article declare that they have no financial conflict of interest with the content of this article.

Supplemental

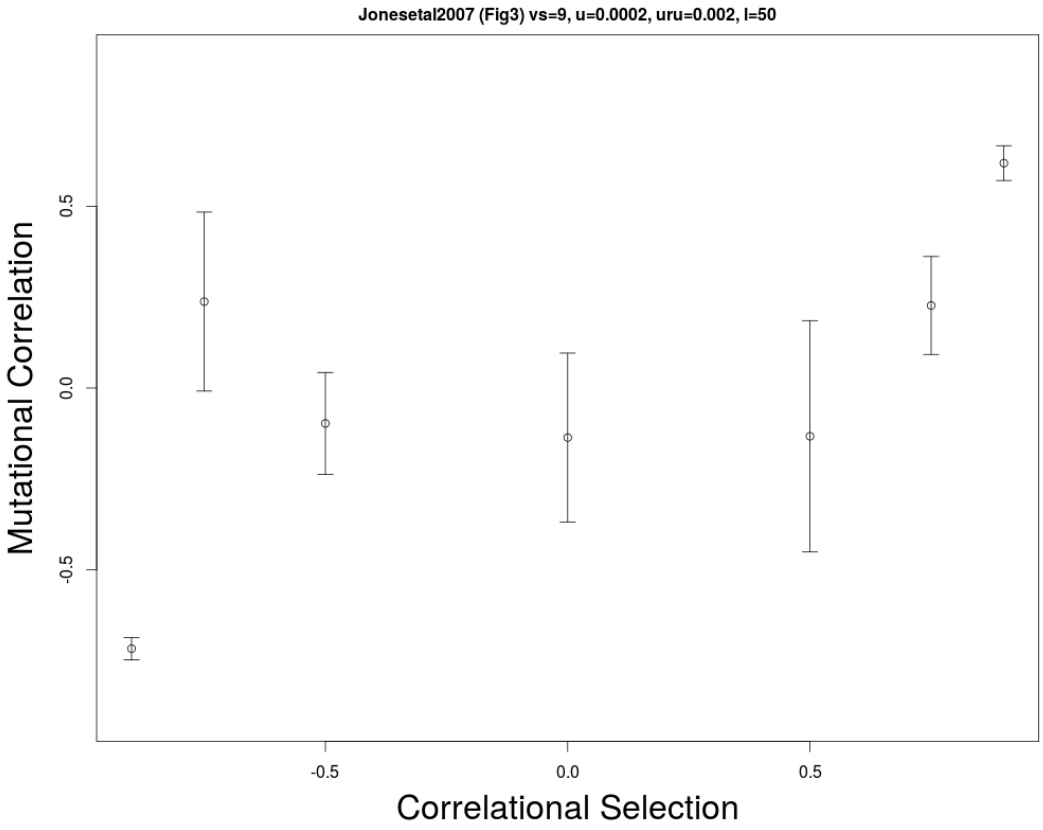

Figure S1: Average mutational correlation ρ_μ values for different values of correlational selection ρ_ω. Parameter values were chosen to match those used in Jones et al. 2007 wherever possible and averages were taken over values from every five generations after burn-in between generation 10,000 and 20,000. Number of loci = 50, Number of traits = 2, $N = 2372$, $\omega^2 = 9$, $\mu = 0.0002$, $\mu_{mutcor} = 0.002$, and $\alpha^2 = 0.05$.

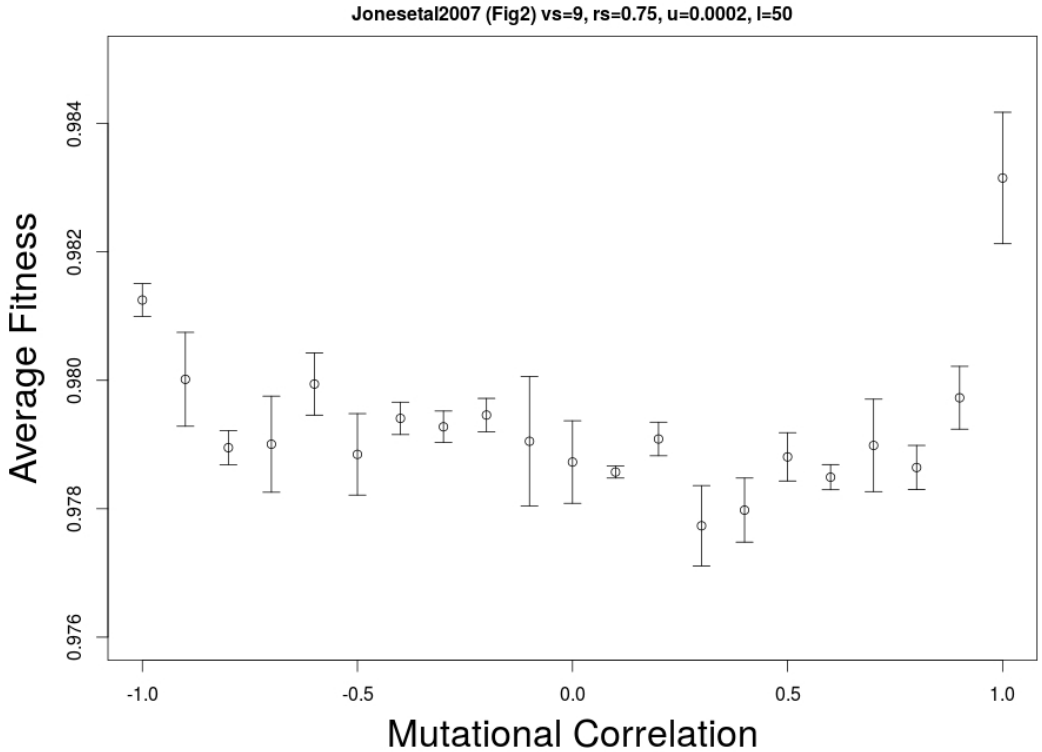

Figure S2: Average fitness for different values of mutational correlation (static). Parameter values were chosen to match those used in Jones et al. 2007 wherever possible and averages were taken over values from every five generations after burn-in between generation 10,000 and 15,000. Number of loci = 50, Number of traits = 2, $N = 2372$, $\omega^2 = 9$, $\rho_\omega = 0.75$, $\mu = 0.0002$, and $\alpha^2 = 0.05$.

References

Albert, A. Y. K., Sawaya, S., Vines, T. H., Knecht, A. K., Miller, C. T., Summers, B. R., Balabhadra, S., Kingsley, D. M., and Schluter, D. (2008). The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution*, 62(1):76–85.

Arnold, S. J. (1992). Constraints on phenotypic evolution. *The American Naturalist*, 140:S85–S107.

Baatz, M. and Wagner, G. P. (1997). Adaptive inertia caused by hidden pleiotropic effects. *Theoretical Population Biology*, 51(1):49–66.

Bomblies, K. and Doebley, J. F. (2006). Pleiotropic effects of the duplicate maize floricaula/leafy genes zfl1 and zfl2 on traits under selection during maize domestication. *Genetics*, 172(1):519–531.

Bult, C. J., Eppig, J. T., Kadin, J. A., Richardson, J. E., Blake, J. A., Airey, M. T., Anagnostopoulos, A., Babiuk, R., Baldarelli, R. M., Baya, M., et al. (2008). The mouse genome database (mgd): mouse biology and model systems. *Nucleic acids research*, 36(suppl 1):D724–D728.

Bürger, R. (1986). Constraints for the evolution of functionally coupled characters: a nonlinear analysis of a phenotypic model. *Evolution*, 40(1):182–193.

Chapman, D. L., Garvey, N., Hancock, S., Alexiou, M., Agulnik, S. I., Gibson-Brown, J. J., Cebra-Thomas, J., Bollag, R. J., Silver, L. M., and Papaioannou, V. E. (1996). Expression of the t-box family genes, tbx1–tbx5, during early mouse development. *Developmental dynamics: an official publication of the American Association of Anatomists*, 206(4):379–390.

Chebib, J. and Guillaume, F. (2017). What affects the predictability of evolutionary constraints using a g-matrix? the relative effects of modular pleiotropy and mutational correlation. *Evolution*.

Cheverud, J. M., Routman, E. J., and Irschick, D. J. (1997). Pleiotropic effects of individual gene loci on mandibular morphology. *Evolution*, 51(6):2006–2016.

Crow, J. F. and Kimura, M. (1964). The theory of genetic loads. *Proc. XI Int. Congr. Genetics*, 2:495–505.

Des Marais, D. L. and Rausher, M. D. (2008). Escape from adaptive conflict after duplication in an anthocyanin pathway gene. *Nature*, 454(7205):762.

Dudley, A. M., Janse, D. M., Tanay, A., Shamir, R., and Church, G. M. (2005). A global view of pleiotropy and phenotypically derived gene function in yeast. *Molecular systems biology*, 1(1):1–11.

Estes, S., Ajie, B. C., Lynch, M., and Phillips, P. C. (2005). Spontaneous mutational correlations for life-history, morphological and behavioral characters in caenorhabditis elegans. *Genetics*, 170(2):645–653.

Force, A., Lynch, M., Pickett, F. B., Amores, A., Yan, Y.-l., and Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, 151(4):1531–1545.

Graham, A. and McGonnell, I. (1999). Limb development: Farewell to arms. *Current biology*, 9(10):R368–R370.

Guillaume, F. and Otto, S. P. (2012). Gene functional trade-offs and the evolution of pleiotropy. *Genetics*, 192:1389–1409.

Guillaume, F. and Rougemont, J. (2006). Nemo:an evolutionary and population genetics programming framework. *Bioinformatics*, 22:2556–2557.

Güldener, U., Münsterkötter, M., Kastenmüller, G., Strack, N., van Helden, J., Lemer, C., Richelles, J., Wodak, S. J., García-Martínez, J., Pérez-Ortín, J. E., et al. (2005). Cygd: the comprehensive yeast genome database. *Nucleic acids research*, 33(suppl 1):D364–D368.

Hahn, M. W. (2009). Distinguishing among evolutionary models for the maintenance of gene duplicates. *Journal of Heredity*, 100(5):605–617.

Hansen, T. F. (2003). Is modularity necessary for evolvability?: Remarks on the relationship between pleiotropy and evolvability. *Biosystems*, 69(2):83–94.

Hansen, T. F. (2006). The evolution of genetic architecture. *Ann. Rev. Ecol. Evol. Syst.*, 37:123–157.

Hodgkin, J. (1998). Seven types of pleiotropy. *Int. J. Dev. Biol.*, 42(3):501–505.

Houle, D. and Fierst, J. (2013). Properties of spontaneous mutational variance and covariance for wing size and shape in Drosophila melanogaster. *Evolution*, 67(4):1116–1130.

Infante, C. R., Mihala, A. G., Park, S., Wang, J. S., Johnson, K. K., Lauderdale, J. D., and Menke, D. B. (2015). Shared enhancer activity in the limbs and phallus and functional divergence of a limb-genital cis-regulatory element in snakes. *Developmental cell*, 35(1):107–119.

Innan, H. and Kondrashov, F. (2010). The evolution of gene duplications: classifying and distinguishing between models. *Nature Reviews Genetics*, 11(2):97.

Jones, A. G., Arnold, S. J., and Bürger, R. (2003). Stability of the g-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution*, 57:1747–1760.

Jones, A. G., Arnold, S. J., and Bürger, R. (2007). The mutation matrix and the evolution of evolvability. *Evolution*, 61(4):727–745.

Jones, A. G., Bürger, R., and Arnold, S. J. (2014). Epistasis and natural selection shape the mutational architecture of complex traits. *Nat. Comm.*, 5:3709–3718.

Juenger, T., Perez-Perez, J. M., Bernal, S., and Micol, J. L. (2005). Quantitative trait loci mapping of floral and leaf morphology traits in Arabidopsis thaliana: evidence for modular genetic architecture. *Evol. Dev.*, 7(3):259–271.

Kenney-Hunt, J. P., Wang, B., Norgard, E. A., Fawcett, G., Falk, D., Pletscher, L. S., Jarvis, J. P., Roseman, C., Wolf, J., and Cheverud, J. M. (2008). Pleiotropic patterns of quantitative trait loci for 70 murine skeletal traits. *Genetics*, 178(4):2275–2288.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution*, 33:402416.

Lande, R. (1980). The genetic covariance between characters maintained by pleiotropic mutations. *Genetics*, 94(1):203–215.

Lynch, M. and Force, A. (2000). The probability of duplicate gene preservation by subfunctionalization. *Genetics*, 154(1):459–473.

Melo, D. and Marroig, G. (2015). Directional selection can drive the evolution of modularity in complex traits. *Proc. Natl. Acad. Sci. U.S.A.*, 112:470–475.

Melo, D., Porto, A., Cheverud, J. M., and Marroig, G. (2016). Modularity: genes, development, and evolution. *Annual review of ecology, evolution, and systematics*, 47:463–486.

Mezey, J. G., Houle, D., and Nuzhdin, S. V. (2005). Naturally segregating quantitative trait loci affecting wing shape of drosophila melanogaster. *Genetics*, 169(4):2101–2113.

Miller, C. T., Glazer, A. M., Summers, B. R., Blackman, B. K., Norman, A. R., Shapiro, M. D., Cole, B. L., Peichel, C. L., Schluter, D., and Kingsley, D. M. (2014). Modular skeletal evolution in sticklebacks is controlled by additive and clustered quantitative trait loci. *Genetics*, 197(1):405–420.

Minguillon, C., Gibson-Brown, J. J., and Logan, M. P. (2009). Tbx4/5 gene duplication and the origin of vertebrate paired appendages. *Proceedings of the National Academy of Sciences*, pages pnas–0910153106.

Muller, H. J. (1936). Bar duplication. *Science*, 83(2161):528–530.

Nagy, O., Nuez, I., Savisaar, R., Peluffo, A. E., Yassin, A., Lang, M., Stern, D. L., Matute, D. R., David, J. R., and Courtier-Orgogozo, V. (2018). Correlated evolution of two copulatory organs via a single cis-regulatory nucleotide change. *Current Biology*, 28(21):3450–3457.

Ohno, S. (1970). Evolution by gene duplication springer. *New York*.

Ohya, Y., Sese, J., Yukawa, M., Sano, F., Nakatani, Y., Saito, T. L., Saka, A., Fukuda, T., Ishihara, S., Oka, S., et al. (2005). High-dimensional and large-scale phenotyping of yeast mutants. *Proceedings of the National Academy of Sciences of the United States of America*, 102(52):19015–19020.

Orr, H. A. (2000). Adaptation and the cost of complexity. *Evolution*, 54(1):13–20.

Pavlicev, M. and Cheverud, J. M. (2015). Constraints evolve: context dependency of gene effects allows evolution of pleiotropy. *Ann. Rev. Ecol. Evol. Syst.*, 46:413–434.

Pavlicev, M., Cheverud, J. M., and Wagner, G. P. (2011). Evolution of adaptive phenotypic variation patterns by direct selection for evolvability. *Proceedings of the Royal Society of London B: Biological Sciences*, 11:rspb20102113.

Pavlicev, M. and Hansen, T. F. (2011). Genotype-phenotype maps maximizing evolvability: Modularity revisited. *Evolutionary Biology*, 38(4):371–389.

Pavlicev, M., Kenney-Hunt, J. P., Norgard, E. A., Roseman, C. C., Wolf, J. B., and Cheverud, J. M. (2008). Genetic variation in pleiotropy: differential epistasis as a source of variation in the allometric relationship between long bone lengths and body weight. *Evolution*, 62(1):199–213.

Perry, G. H., Dominy, N. J., Claw, K. G., Lee, A. S., Fiegler, H., Redon, R., Werner, J., Villanea, F. A., Mountain, J. L., Misra, R., et al. (2007). Diet and the evolution of human amylase gene copy number variation. *Nature genetics*, 39(10):1256.

Petit, F., Sears, K. E., and Ahituv, N. (2017). Limb development: a paradigm of gene regulation. *Nature Reviews Genetics*, 18(4):245.

Polster, R., Petropoulos, C. J., Bonhoeffer, S., and Guillaume, F. (2016). Epistasis and pleiotropy affect the modularity of the genotype–phenotype map of cross-resistance in hiv-1. *Molecular Biology and Evolution*, 33(12):3213–3225.

Porto, A., Schmelter, R., VandeBerg, J. L., Marroig, G., and Cheverud, J. M. (2016). Evolution of the genotype-to-phenotype map and the cost of pleiotropy in mammals. *Genetics*, 204(4):1601–1612.

Qian, W., Liao, B.-Y., Chang, A. Y.-F., and Zhang, J. (2010). Maintenance of duplicate genes and their functional redundancy by reduced expression. *Trends in Genetics*, 26(10):425–430.

Rastogi, S. and Liberles, D. A. (2005). Subfunctionalization of duplicated genes as a transition state to neofunctionalization. *BMC evolutionary biology*, 5(1):28.

Rice, G. and Rebeiz, M. (2019). Evolution: How many phenotypes do regulatory mutations affect? *Current Biology*, 29(1):R21–R23.

Rueffler, C., Hermisson, J., and Wagner, G. P. (2012). Evolution of functional specialization and division of labor. *Proceedings of the National Academy of Sciences*, 109(6):E326–E335.

Smith, S. D. (2016). Pleiotropy and the evolution of floral integration. *New Phytologist*, 209(1):80–85.

Sönnichsen, B., Koski, L., Walsh, A., Marschall, P., Neumann, B., Brehm, M., Alleaume, A.-M., Artelt, J., Bettencourt, P., Cassin, E., et al. (2005). Full-genome rnai profiling of early embryogenesis in caenorhabditis elegans. *Nature*, 434(7032):462–469.

Stern, D. L. (2000). Perspective: evolutionary developmental biology and the problem of variation. *Evolution*, 54(4):1079–1091.

Wagner, G. (1984). Coevolution of functionally constrained characters: prerequisites for adaptive versatility. *BioSystems*, 17(1):51–55.

Wagner, G. P. (1989). Multivariate mutation-selection balance with constrained pleiotropic effects. *Genetics*, 122(1):223–234.

Wagner, G. P. and Altenberg, L. (1996). Perspective: complex adaptations and the evolution of evolvability. *Evolution*, 50(3):967–976.

Wagner, G. P. and Zhang, J. (2011). The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms. *Nat. Rev. Genet.*, 12(3):204–213.

Wang, Z., Liao, B. Y., and Zhang, J. Z. (2010). Genomic patterns of pleiotropy and the evolution of complexity. *Proc. Natl. Acad. Sci. U.S.A.*, 107:18034–18039.

Welch, J. J. and Waxman, D. (2003). Modularity and the cost of complexity. *Evolution*, 57(8):1723–1734.

Zhang, J. (2003). Evolution by gene duplication: an update. *Trends in ecology & evolution*, 18(6):292–298.

Zhang, X. S. and Hill, W. G. (2002). Joint effects of pleiotropic selection and stabilizing selection on the maintenance of quantitative genetic variation at mutation-selection balance. *Genetics*.