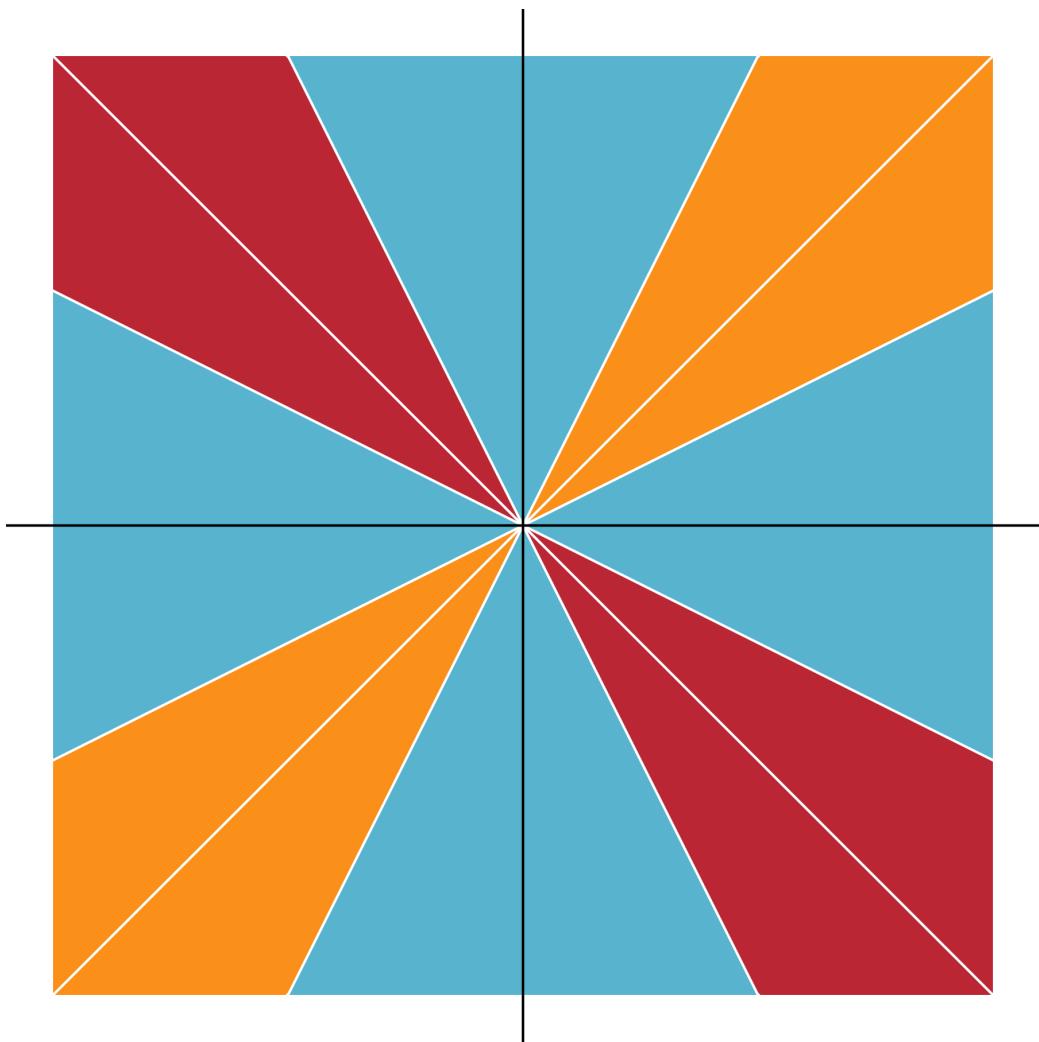


Diogo Amaral R Melo

Evolução da covariação genética em caracteres complexos: interação entre o mapa genótipo-fenótipo e seleção natural



São Paulo
2018

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Evolução da covariação genética em caracteres complexos: interação entre o mapa genótipo-fenótipo e seleção natural

Evolution of genetic covariation in complex
traits: an interplay between the
genotype-phenotype map and natural selection

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ção de Título de Doutor em Ciências, na Área
de Biologia (Genética).

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Para Tomás e Rita.

A human being should be able to change a diaper,
plan an invasion, butcher a hog, conn a ship,
design a building, write a sonnet, balance accounts,
build a wall, set a bone, comfort the dying,
take orders, give orders, cooperate, act alone,
solve equations, analyze a new problem, pitch manure,
program a computer, cook a tasty meal,
fight efficiently, die gallantly.
Specialization is for insects.

Robert A. Heinlein

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Essa tese é pra vocês, espero que gostem.

Resumo

Caracteres complexos são aqueles determinados por muitos genes e que apresentam variação contínua. Em uma população, a variação herdável dos caracteres complexos não é independente, e pares de caracteres podem ser mais ou menos correlacionados entre si. O nível e o padrão da associação entre caracteres determina como o fenótipo da população se comporta perante os processos evolutivos. A associação entre caracteres pode tanto facilitar a evolução em algumas direções do espaço fenotípico quanto restringir a evolução em outras, pois caracteres mais associados entre si tendem a evoluir de forma conjunta. O padrão de associação entre caracteres pode ser representado pela matriz de covariância genética aditiva, que descreve o padrão variacional resultante da interação do mapa genótipo-fenótipo e de todos os processos de desenvolvimento que levam desde a informação contida no material genético até o indivíduo. Tanto o mapa genótipo-fenótipo quanto o padrão de covariação genético também apresentam variação herdável, e portanto podem ser alterados pelos processos evolutivos e mudar entre gerações. Esse processo estabelece uma interação de mão dupla entre evolução e covariação, na qual a covariação afeta o resultado dos processos evolutivos e os processos evolutivos afetam a covariação. Nesta tese, nós exploramos como os efeitos genéticos interagem para formar o padrão de covariação, e como esses efeitos e covariação evoluem sob seleção natural. Para isso, nós trabalhamos com três populações experimentais de camundongos que foram sujeitas a regimes de seleção artificial e, utilizando diferentes tipos de caracteres, procuramos entender como a covariação se estabelece e como ela é afetada pela seleção. No primeiro experimento, estudamos o padrão de covariação de caracteres crânicos em linhagens selecionadas para aumento e diminuição do tamanho corporal, e observamos que a seleção para tamanho altera os caracteres do crânio e a covariação entre eles. A seleção direcional diminui a variação total do crânio, mas também aumenta a proporção de variação na direção de seleção, potencialmente facilitando uma nova resposta seletiva na mesma direção. Esse resultado

implica que a variação presente em uma população pode ser moldada pela sua história evolutiva de forma adaptativa. No segundo experimento utilizamos uma população intercruzada, criada a partir linhagens selecionadas para aumento e diminuição do tamanho corporal, para identificar regiões genômicas envolvidas na determinação da curva de crescimento. Utilizando estimativas dos efeitos genotípicos nos fenótipos de crescimento, nós pudemos prever os fenótipos das linhagens ancestrais utilizando apenas informação da população intercruzada, e também construir estimativas de qual seria a covariação entre os caracteres de crescimento para cada tipo de efeito genético. Além disso, relacionamos a distribuição dos efeitos genéticos com a história evolutiva da população, mostrando que tanto a seleção quanto restrições internas do desenvolvimento interagem para determinar a distribuição de efeitos genéticos e, portanto, a covariação. No terceiro experimento, utilizamos seis linhagens de camundongos, que haviam sido selecionadas para alterações na curva de crescimento, para formar uma população intercruzada. Essa população apresentava uma enorme variação na sua curva de crescimento, e, utilizando técnicas de mapeamento genético, nós identificamos regiões genômicas envolvidas na determinação dessa variação fenotípica. Também desenvolvemos, para criar uma expectativa para a distribuição de efeitos genéticos nessa população, um modelo de simulação computacional da evolução dos efeitos genotípicos sob seleção. Os efeitos genéticos na população intercruzada apresentam um padrão mais complexo que o das simulações, e encontramos uma combinação de efeitos genéticos com padrões diferentes que interagem para gerar a covariação genética presente na população. Por fim, apresentamos uma revisão sobre a evolução da covariação genética e discutimos as consequências macroevolutivas das questões abordadas nos outros capítulos.

Palavras-chave: genética quantitativa, mapeamento de QTL, seleção direcional

Abstract

Complex traits are defined as traits that are determined by many genes and that show continuous variation. In a population, the heritable variation of complex traits is not independent, and pairs of traits might be more or less correlated. The level and pattern of the association between traits determine how the phenotype of the population behaves when faced with evolutionary forces, like natural selection and genetic drift. The association between traits can both facilitate evolutionary change in some directions of the phenotype space and hinder change in other directions because tightly associated traits tend to evolve together. The pattern of association among traits can be represented by the additive genetic covariance matrix. This matrix describes the variational pattern that is the result of the interplay between the genotype-phenotype map and development, which together lead from the genetic information to the formation of the individual. Both the genotype-phenotype map and the genetic covariation also show heritable variation, and so are able to evolve and change between generations. This process establishes a feedback between evolution and covariation, in which covariation affects the outcome of the evolutionary process and is also shaped by evolution. In this thesis, we explore how genetic effects interact to create patterns of covariation, and how these effects and covariation change under natural selection. In order to do this, we use three experimental mice populations that were subjected to artificial selection regimes, and, using several types of complex traits, we study how covariation is established and how it evolves. In the first experiment, we use the covariation pattern of cranial traits measured in mice strains selected for the increase and decrease of body size. In these strains, we see that size selection altered the means of the cranial traits and the covariation between them. Directional selection reduces the total amount of genetic information, but in a non-uniform way. Some directions in phenotype space lose more variation than others, and, counter-intuitively, the direction of selection loses less variation. This leads to an increase in the proportion

of variation that is in the direction of selection, potentially facilitating future evolutionary change in the same direction. This result shows that the covariation pattern in a population is shaped by its evolutionary history and can be adaptive. In the second experiment, we use an intercross population, created with two inbred mouse strains that were selected for increase and decrease in weight, to identify genomic regions involved in determining the growth curve of the individuals. Using estimates of the genetic effects on the growth traits, we were able to predict the phenotypes of the ancestral strains using only information from the intercross. We were also able to partition the genetic covariation into the contributions due to different types of genetic effects. We interpret the distribution of genetic effects in light of the evolutionary history of the population and show that the distribution of genetic effects, and of genetic covariation, is a consequence of the interaction between selection and development. In the third experiment, we create an intercross using six inbred mice strains that had been selected for different changes in their growth curve. This intercross shows large variation in growth curves, and, using genetic mapping techniques, we identify genomic regions involved in producing this phenotypic variation. To create an expectation for the distribution of genetic effects in this population, we develop a computer simulation model for the evolution of genetic effects under directional selection. The genetic effects in the population are more complex than in the simulation model, and we find that the genetic covariation between growth traits is created by the interaction among several different kinds of genetic effects. Finally, we present a review on the evolution of genetic covariation and discuss the macroevolutionary consequences of the themes we explore in the other chapters.

Keywords: quantitative genetics, QTL mapping, directional selection

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Capítulo 1

Introdução

1.1 Motivação

Esta tese surgiu da vontade de entender como os processos internos ao organismo que produzem variação em uma população interagem com os processos evolutivos para dar origem à diversidade que observamos na natureza. Nós procuramos estudar como a covariância genética se estabelece e como ela evolui, e situamos essas questões dentro do contexto da macroevolução de caracteres quantitativos. Para isso, nos valemos da teoria de genética quantitativa na sua encarnação mais moderna, aliada a teoria de evolução e das técnicas de mapeamento de loci de caracteres quantitativos. Essa combinação permite entender as origens e prever as consequências das restrições genéticas na evolução dos caracteres, e esmiuçar a arquitetura genética por trás dessas restrições. Nesta introdução, vamos revisar rapidamente a teoria de genética quantitativa evolutiva e discutir a estrutura geral da tese. Neste capítulo vamos utilizar as citações com moderação, de forma a deixar o texto mais fluido. A maior parte do contexto teórico pode ser encontrado em livros básicos de evolução e genética, como Barton et al. (2007); Falconer e Mackay (1996); Lynch e Walsh (1998). Algumas questões mais avançadas podem ser encontradas em Bürger (2000); Rice (2004). Argumentos que surgiram em artigos específicos e são mais associados a essa referência serão apontados no texto.

1.2 Variação genética e evolução

A variação fenotípica presente em uma população é fundamental para que a evolução natural possa ocorrer. Em última instância, a variação fenotípica que está disponível para que a evolução aconteça é consequência da variação genética da população¹. Essa variação genética se dá no nível do DNA, com variantes discretas que segregam na população. A informação genética contida nessas variantes é interpretada pelo processo de desenvolvimento, e por meio de um sistema extremamente complexo de interações, a cada geração um novo indivíduo é formado a partir da informação no código genético. Mendel descreveu as leis que regem a herança dessas variantes discretas estudando caracteres de herança discreta e simples, ervilhas verdes ou amarelas, lisas ou rugosas. Mesmo esses caracteres simples, cuja variação é determinada por um único locus do genoma, tem por trás de si um intrincado processo de desenvolvimento. Se para entender a evolução de qualquer caráter, sobretudo caracteres complexos, fosse necessário um modelo do processo que leva à sua formação, o estudo de evolução seria uma iniciativa inviável. Felizmente, essa compreensão total do organismo não é necessária. Para o estudo de evolução, basta um modelo de como os organismos mudam. Somente a ligação entre variação genética e mudança fenotípica importa, não o mecanismo por trás da formação do fenótipo. Se um gene está envolvido no desenvolvimento de um caráter mas não apresenta variação genética, esse gene não é importante para entender a evolução do caráter.

1.2.1 Genética quantitativa

A grande maioria dos caracteres interessantes do ponto de vista evolutivo tem uma base genética complexa. Tamanho corporal, sucesso reprodutivo, coloração, forma, são todos caracteres cuja variação é determinada por muitos genes. Por um lado, isso faz com que entender a arquitetura genética da variação desses caracteres seja uma tarefa complicada. Por outro lado, essa base complexa garante que esses caracteres apresentem um padrão de variação contí-

¹A rigor, variação genética não é estritamente necessária, apenas variação *herdável*. A herança cultural, por exemplo, pode ser estudada com as ferramentas da evolução. Apesar disso, em populações naturais, a maior parte da variação herdável é genética.

nuo, e portanto possam ser estudados utilizando as ferramentas da genética quantitativa. A variação contínua que encontramos em caracteres complexos se deve a dois fatores: a base poligênica e a variação ambiental a que os fenótipos estão sujeitos. Quando vários genes estão envolvidos na determinação de um caráter, a junção dos efeitos de herança particulada de cada uma das variantes em cada um dos locus acaba por definir tantas classes de fenótipos que a variação pode ser tratada como contínua. Além disso, se assumirmos que cada gene contribui de forma aditiva ao fenótipo, o teorema do limite central diz que a distribuição do caráter na população pode ser aproximado por uma distribuição Gaussiana (também chamada de distribuição normal). Na prática, a grande maioria dos caracteres de interesse evolutivo podem ser aproximados por uma distribuição normal², ou transformados trivialmente para uma distribuição normal³. Além disso, existem diferenças ambientais no desenvolvimento de um caráter. Mesmo uma população de indivíduos geneticamente idênticos irá apresentar alguma variação em seus caracteres. Essa variação ambiental contribui para o aumento do número de fenótipos possíveis e para a possibilidade de descrever a variação como contínua (Fig. 1.1).

A ideia que a variação de um fenótipo pode ser expressa como a soma de vários efeitos aditivos independentes, proposta por Fisher (1918), é central para a genética quantitativa. A primeira distinção é entre os componentes devidos a diferenças genéticas entre os indivíduos e os componentes devidos a diferenças não genéticas (ambientais) da variação em uma população. Para entender como o fenótipo é composto, podemos definir G como a média do fenótipo dos indivíduos que tem um determinado genótipo e E como o desvio devido ao ambiente. O fenótipo P do indivíduo então é definido como:

$$P = G + E \quad (1.1)$$

²Curiosamente, a observação de que a distribuição Gaussiana era tão ubíqua e útil na biologia evolutiva foi feita inicialmente por um primo de Darwin, Francis Galton, em 1869.

³Caracteres contínuos que são restritos a valores positivos, como distâncias ou pesos, podem apresentar uma distribuição log normal, em que o log das medidas tem distribuição normal. Essa transformação implica que os genes tem efeitos multiplicativos no fenótipo, e a transformação log transforma essas multiplicações em adições, levando à distribuição normal após a transformação.

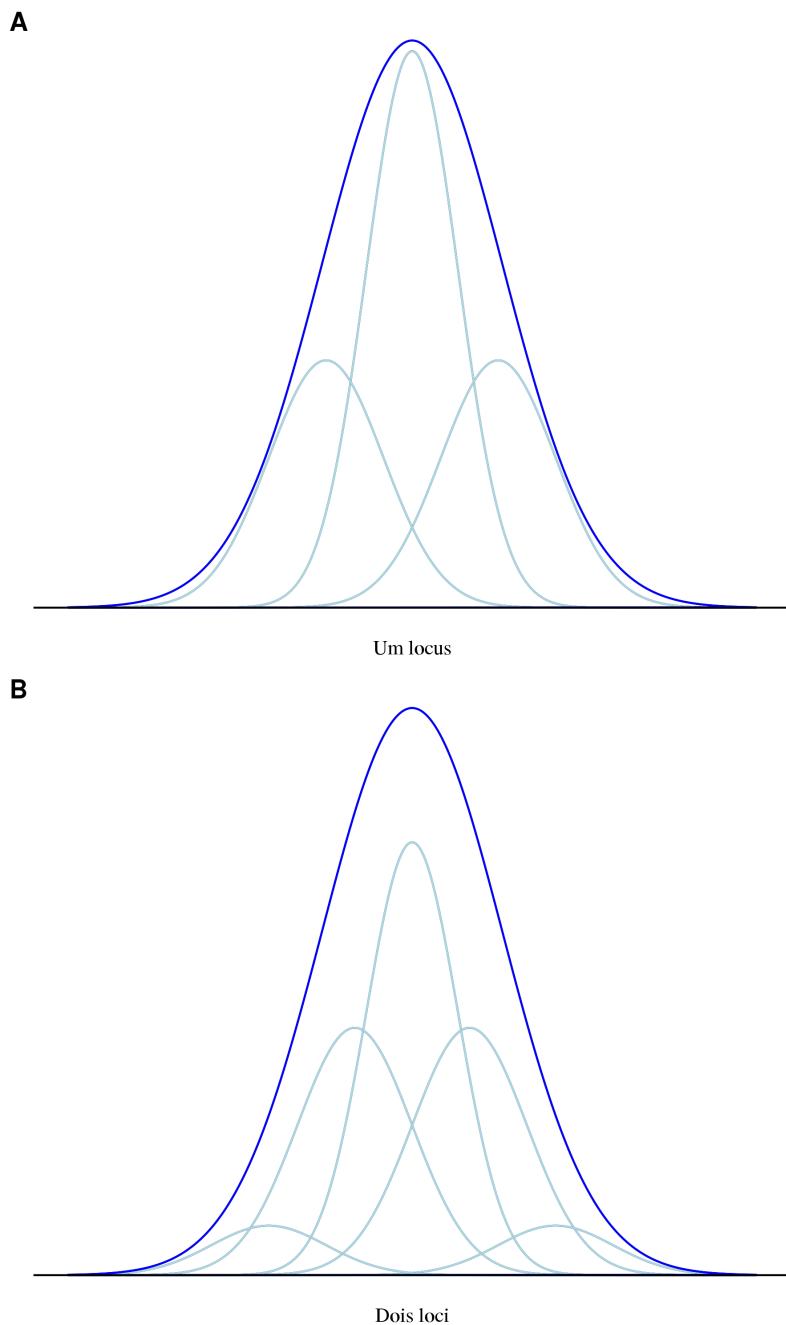


Figura 1.1: Mesmo com poucos loci, o modelo aditivo e variação ambiental são capazes de gerar distribuições fenotípicas muito próximas à Gaussiana, desde que a diferença entre classes genotípicas seja da mesma escala da variação dentro de cada classe. A) Um loci aditivo com dois alelos de mesma frequência e a variação ambiental dentro de cada genótipo. As linhas finas mostram a variação dentro de cada classe, e a linha grossa, a distribuição na população. B) Dois loci com dois alelos cada, com efeitos fenotípicos equivalentes. Adaptado de Barton et al. (2007).

Como G é definido como a média de P , a média de E deve ser zero. Se a distribuição de efeitos ambientais for aproximadamente normal e se não existir interação entre E e G , ou seja, se a variação ambiental for a mesma para todos os genótipos, um genótipo pode ser descrito apenas pelo seu valor genotípico G e pela sua variância ambiental V_E . Se a população em questão é formada de indivíduos sexuados, o valor genotípico de um indivíduo será composto pela soma das contribuições dos gametas parentais. Suponha que para os dois loci que afetam um dado caráter o indivíduo tenha recebido um gameta A_1B_1 do pai e um gameta A_2B_1 da mãe. Assim, o genótipo do indivíduo é $A_1A_2B_1B_1$, heterozigoto no locus A e homozigoto no locus B. Dentro de um modelo aditivo, no qual os efeitos de cada alelo são somados, o fenótipo do indivíduo seria $P = \alpha_{A_1} + \alpha_{A_2} + 2\alpha_{B_1} + E$, onde os α representam as contribuições aditivas de cada alelo, que juntos compõem o valor genotípico do indivíduo.

Naturalmente, o modelo puramente aditivo é uma simplificação enorme, e, na prática, existem dois tipos de interações importantes entre alelos que podem contribuir com o valor genotípico: interações de dominância, que acontecem entre alelos do mesmo locus, e interações epistáticas, que acontecem entre alelos em loci diferentes. Dominância acontece quando os efeitos dos alelos em um locus não são simplesmente aditivos, ou, em outras palavras, quando o heterozigoto não tem valor genotípico intermediário aos dois homozigotos correspondentes. Já epistasia acontece quando o efeito de um alelo em um locus depende do estado de outro locus. Ambas formas de interação são extremamente comuns e contribuem de forma decisiva para a mudança evolutiva, principalmente epistasia, como veremos mais adiante.

Dentro do formalismo da genética quantitativa, as interações entre alelos e loci podem ser incluídas na formação do valor genotípico, também de forma aditiva. Então, a equação para o fenótipo de um indivíduo se torna:

$$P = G + E = A + D + I + E \quad (1.2)$$

na qual nós decomponemos o valor genotípico em seus termos devidos aos efeitos aditivos

(A), de dominância (D) e epistáticos (I , interação). O termo A também é chamado de valor de acasalamento, e, além de ser a soma das contribuições aditivas de cada alelo, este termo tem uma interpretação simples que não faz referência à base genética do fenótipo. Para um dado fenótipo, o valor de acasalamento de um indivíduo pode ser definido como duas vezes a diferença entre a média do fenótipo da sua prole com parceiros escolhidos ao acaso na população e a média do fenótipo da população como um todo. A diferença é dobrada pois o indivíduo contribui com metade do valor genotípico dos seus filhos, a outra metade vindo ao acaso da população.

1.2.2 Componentes da variação fenotípica

Apesar de conceitualmente importantes, são raras as ocasiões em que temos condição de medir os valores dos componentes genotípicos de um indivíduo. Estimar esses valores envolveria conhecer o genótipo de todos os indivíduos de uma população em todos os locus que fossem de alguma forma envolvidos na variação do fenótipo, uma tarefa praticamente impossível. A importância prática desse esquema de decomposição fica mais clara quando consideramos a variação na população como um todo. A decomposição da variância da população nos seus componentes devido a efeitos aditivos, de dominância e todos os outros componentes do fenótipo pode ser feita sem nenhuma referência à base genética específica do fenótipo. Supondo que a variação genética e ambiental são independentes, podemos expressar a variação fenotípica (V_P) como a soma das variâncias genéticas e ambientais:

$$V_P = V_G + V_E \quad (1.3)$$

Do mesmo modo, a variância dos valores genotípicos pode ser subdividida nos componentes gerados pelos diferentes tipos de efeitos genéticos:

$$V_G = V_A + V_D + V_I \quad (1.4)$$

O componente da variância devido a diferenças nos valores de acasalamento (V_A) é chamado de variância genética aditiva, e tem um papel fundamental na biologia evolutiva. O componente devido a interações epistáticas, V_I , também pode ser subdividido em diferentes tipos de interação entre dois loci: entre componentes aditivos (V_{AA}), entre componentes aditivos e de dominância (V_{AD}), entre componentes de dominância (V_{DD}), e mesmo componentes de grau mais alto, entre 3 ou mais loci. Essa subdivisão fornece uma ferramenta poderosa para o estudo da variação e evolução em populações naturais.

Cada um desses componentes pode ser estimado a partir da semelhança entre indivíduos relacionados em uma população. Por exemplo, a similaridade entre pais e filhos, fundamental na herança de caracteres e para a evolução, pode ser usada para estimar a variância do valores de acasalamento. No caso mais simples, no modelo aditivo, o fenótipo de um indivíduo (filho) é dado por $P_f = A + E_f$. O componente aditivo pode ser subdividido em duas partes, as contribuições do pai e da mãe, então $P_f = A_1 + A_2 + E_f$. Já o fenótipo do pai pode ser escrito como $P_p = A_1 + A_3 + E_p$, onde A_1 representa a porção do valor de acasalamento compartilhada com o filho e A_3 a porção independente do filho. A covariância entre pai e filho então é $cov(P_f, P_p) = cov(A_1 + A_2 + E_f, A_1 + A_3 + E_p)$. A covariância da soma de fatores independentes pode ser escrita como a soma das covariâncias par a par, então $cov(P_f, P_p) = cov(A_1, A_1) + cov(A_1, A_3) + cov(A_2, A_1) + \dots + cov(E_f, E_p)$. Como todos os componentes são independentes por construção, o único termo que contribui para a covariância entre pais e filhos é $cov(A_1, A_1) = var(A_1)$. Como a variância aditiva total é a soma das contribuições paternas e maternas $V_A = var(A_1) + var(A_2)$, e como as contribuições paternas e maternas são equivalentes, a covariância entre pais e filhos é a metade da variância aditiva total ($cov(P_f, P_p) = var(A_1) = \frac{1}{2}V_A$). A partir de deduções semelhantes, podemos estimar vários dos componentes da variação genotípica a partir da covariância entre indivíduos relacionados. Por exemplo, a variância de dominância pode ser estimada comparando a covariância entre irmãos completos e pais e filhos, pois irmãos podem compartilhar genótipos, enquanto pais, em geral, não compartilham genótipos com os filhos. Então, na presença de dominância, a covariância entre irmãos é maior que a covariância entre pais e filhos.

1.2.3 Herdabilidade e a resposta à seleção

Para entender como a variação genética influencia a resposta à seleção entre duas gerações, podemos utilizar uma regressão linear entre a média do fenótipo dos casais que se reproduziram e o fenótipo dos filhos e imaginar qual seria a resposta na geração dos filhos a um evento de seleção na geração dos pais. No caso mais simples, de seleção por truncamento, podemos calcular a nova média de pais e filhos após um evento de seleção no qual apenas casais com média do fenótipo acima de um limiar de seleção deixam descendentes. Na figura 1.2 podemos ver que a mudança na média na geração dos filhos será dada pelo produto entre a inclinação da reta de regressão entre pais e filhos e a mudança na média na geração dos pais. A solução de mínimos quadrados para a inclinação da reta de regressão é dada pela covariância entre a variável preditora e a resposta dividida pela variância da preditora. Já vimos que a covariância entre pais e filhos é dada pela metade da variância aditiva, e a covariância entre a média dos pais e filhos tem o mesmo valor, assumindo que a covariância entre pais e filhos e entre mães e filhos é igual. Já o denominador, a variância na média dos pais, pode ser calculado como:

$$\text{var}\left(\frac{P_{\text{pais}} + P_{\text{mães}}}{2}\right) = \frac{\text{var}(P_{\text{pais}}) + \text{var}(P_{\text{mães}})}{4} = \frac{V_P}{2} \quad (1.5)$$

onde assumimos que a variância de pais e mães é igual. Então, a inclinação da reta de regressão é igual à razão entre a variância aditiva e a variância fenotípica total. Essa razão, a proporção da variação fenotípica que é devido à variação aditiva, recebe um tratamento especial dentro da genética quantitativa, recebendo o nome de herdabilidade ($h^2 = \frac{V_A}{V_P}$).⁴

Esse tratamento de regressão nos mostra como a herdabilidade é especialmente importante para entender a resposta evolutiva, pois é ela que determina como uma mudança na média dos pais se traduz numa mudança evolutiva na média dos filhos. Essa relação é dada pela equação do criador, que relaciona a mudança em um fenótipo de uma geração para outra (a resposta à seleção, R) com a mudança dentro de uma geração devido a um episódio de seleção (o diferencial de seleção, S):

⁴O quadrado faz parte do símbolo, e se refere ao fato da herdabilidade ser uma razão de variâncias, e portanto numa escala quadrática.

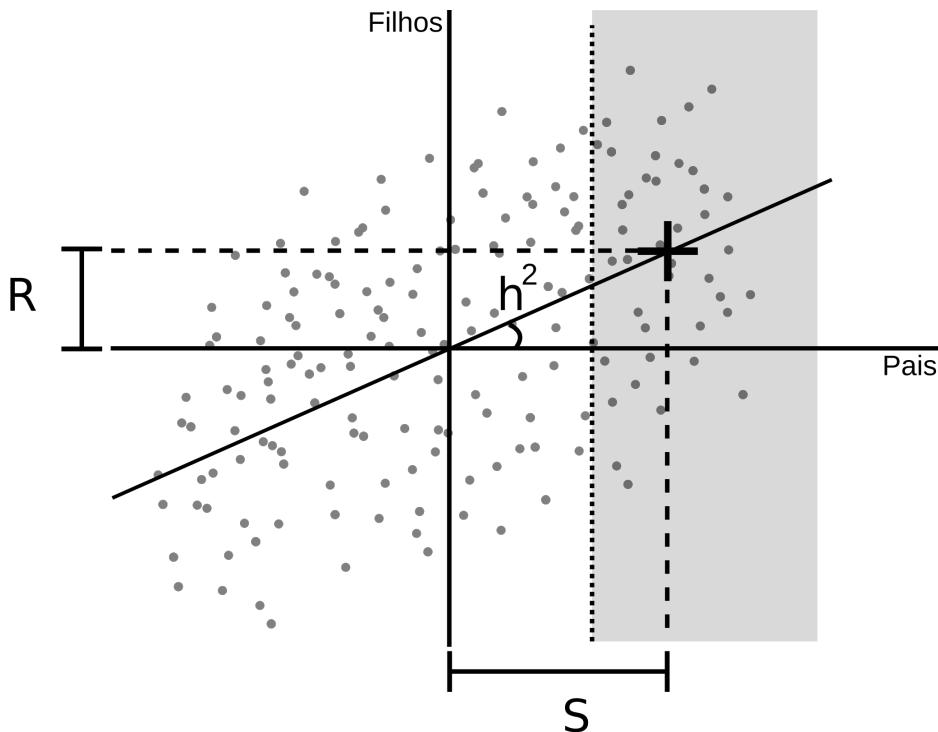


Figura 1.2: Regressão do fenótipo dos filhos na média do fenótipo dos pais permite prever a resposta à seleção direcional, em que a média da população em uma geração é alterada por um evento de seleção. Neste caso, aplicamos um limiar de seleção na geração parental, e apenas casais com média do fenótipo maior que o limiar (na região sombreada) deixam descendentes, a cruz escura marca a nova média após a seleção. A mudança na média da geração dos pais após o evento de seleção é dada pelo diferencial de seleção S . Essa mudança na média causa uma resposta evolutiva (R) na média da geração dos filhos que pode ser predita utilizando uma regressão linear. A inclinação da regressão é dada pela covariância entre pais e filhos e a variância fenotípica na geração parental. Em condições bastante gerais, a inclinação da reta de regressão pode ser estimada como a razão entre a variância aditiva e a variância fenotípica, a herdabilidade (h^2).

$$R = h^2 S = \frac{V_A}{V_P} S \quad (1.6)$$

A intensidade de seleção é medida pelo diferencial de seleção, e quanto maior a mudança na média dentro da geração dos pais, maior será a mudança entre gerações. Além disso, a equação do criador nos mostra que a resposta à seleção depende não só da intensidade de seleção, mas também depende fundamentalmente da presença de variação aditiva. Sem variação aditiva, sem herdabilidade, não há como haver resposta na próxima geração, e quanto maior a herdabilidade maior será a resposta à seleção.

1.2.4 Resposta à seleção multivariada

Se estamos interessados em fenótipos complexos, compostos de muitos caracteres, além de quantificar a variância individual de cada um, podemos também levar em conta a interação entre eles. De forma análoga à variância, a covariância mede a variação conjunta de dois caracteres. A matriz de covariância genética aditiva (matriz G) representa na diagonal as variâncias dos valores de acasalamento de cada caráter, e fora da diagonal a covariância entre os valores de acasalamento de caracteres diferentes. Já a matriz de covariância fenotípica (matriz P) reúne as variância e covariâncias entre os fenótipos da população.

Usando essas matrizes é possível escrever o análogo multivariado da equação do criador, a equação de resposta à seleção multivariada de Lande (1979), que tem essencialmente a mesma forma:

$$\Delta\bar{z} = GP^{-1}S = G\beta \quad (1.7)$$

Nesse caso, tanto a resposta à seleção ($\Delta\bar{z}$) quanto o diferencial de seleção (S) são representados por vetores, sendo que cada componente representa um caráter analisado. Frequentemente, o termo $P^{-1}S$ é representado por β , o gradiente de seleção. O uso do gradiente de seleção é conveniente por algumas razões. Primeiro, o vetor β possui uma interpretação geo-

métrica simples, representando a direção fenotípica de maior aumento da aptidão⁵; segundo, o gradiente de seleção já desconta as covariâncias fenotípicas e permite inferir quais caracteres estão efetivamente sob seleção.⁶

Se as matrizes P e G forem diagonais, ou seja, se todas as covariâncias entre valores de acasalamento e entre fenótipos forem nulas, a equação de Lande se resume a várias instâncias da equação do criador. Porém, caso as covariâncias não sejam nulas, a equação de Lande prevê uma resposta indireta à seleção mesmo em caracteres que não estão sob seleção, caso eles sejam correlacionados com caracteres que estão sob seleção. Para entender esse efeito indireto, podemos considerar o caso com dois caracteres, em que a equação de Lande é:

$$G\beta = \begin{pmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{pmatrix} \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} = \begin{pmatrix} G_{11}\beta_1 + G_{12}\beta_2 \\ G_{21}\beta_1 + G_{22}\beta_2 \end{pmatrix} = \begin{pmatrix} \Delta z_1 \\ \Delta z_2 \end{pmatrix} = \Delta z \quad (1.8)$$

Os termos $G_{11}\beta_1$ e $G_{22}\beta_2$ representam a resposta à seleção direta em cada caráter. Mas além dos termos diretos, temos também os termos indiretos $G_{12}\beta_2$ na resposta do caráter z_1 e $G_{21}\beta_1$ na resposta do caráter z_2 , que dependem da seleção no outro caráter e da covariância genética entre caracteres. A presença de respostas indiretas a um gradiente de seleção faz com que a resposta observada (Δz) não tenha a mesma direção do gradiente de seleção. Esse desvio da resposta evolutiva em relação à seleção direcional é um tipo de restrição genética, e pode ter consequências importantes na diversificação. Por exemplo, a direção de maior variação genética cria uma direção preferencial de mudança evolutiva, ao longo da qual a resposta evolutiva é mais eficiente. Esta direção do espaço fenotípico é chamada de linha genética de menor resistência evolutiva (Schluter 1996). Seleção ao longo dessa linha resulta na maior magnitude de resposta seletiva, e seleção ao longo de outras direções é desviada para a direção de menor resistência⁷. A influência dessa restrição causada pela linha de menor resistência

⁵Dai o nome **gradiente**, que no cálculo diferencial é definido como o vetor de derivadas que aponta a direção de maior mudança em uma superfície multivariada.

⁶Isso só é valido caso todos os caracteres relevantes para a aptidão estejam inclusos na análise.

⁷Desde que a direção de seleção não seja ortogonal à linha de menor resistência.

poder ser observada mesmo na diferença entre gêneros (Marroig e Cheverud 2005). Além disso, grupos de caracteres que possuam covariâncias altas entre si podem influenciar a resposta um do outro, pois caso um dos caracteres esteja sob seleção direcional, os outros irão sofrer respostas indiretas. Por isso, grupos de caracteres ligados por covariâncias genéticas tendem a evoluir de forma conjunta.

1.3 Estrutura da variação genética

A influência da covariação genética na resposta à seleção implica que conhecer o padrão e intensidade da covariância genética é fundamental para o estudo de evolução. O padrão de covariância genética se refere à organização da covariação, quais caracteres são mais relacionados, quais grupos têm covariâncias positivas ou negativas, quais caracteres compõem um grupo de covariâncias altas, etc. Já a intensidade se refere à magnitude total das covariâncias. Como as covariâncias são medidas na escala dos caracteres, é comum utilizar uma medida de associação padronizada, que permita a comparação da intensidade de associação entre caracteres de tamanhos e escalas de variação diferentes. A medida usual é a correlação entre caracteres, definida como a covariância dividida pelo produto dos desvios padrões dos caracteres em questão. Tanto o padrão quanto a intensidade das correlações genéticas são temas centrais desta tese.

O padrão de correlação genético é principalmente definido pela arquitetura genética dos caracteres. Genes pleiotrópicos, que afetam a variação em mais de um caráter, e desequilíbrio de ligação, a herança conjunta de alelos específicos, contribuem para a covariância genética e provocam respostas correlacionadas. Em uma parcela significativa dos sistemas biológicos, da expressão gênica à morfologia, a organização da covariação genética se dá de forma modular. Um padrão de correlação é dito modular quando os caracteres se encontram organizados em conjuntos de caracteres mais correlacionados entre si do que com o restante do organismo. A percepção da importância da organização modular em sistemas morfológicos se deve em

grande parte a Olson e Miller (1958). Neste livro, os autores argumentam que grupos de caracteres altamente correlacionados são um reflexo do seu envolvimento no desempenho de uma função específica e de sua associação via desenvolvimento, compartilhando uma origem embriológica ou sendo formados pelas mesmas vias de desenvolvimento. A ideia de organização modular permeia toda esta tese e serve de arcabouço teórico importante para a nossa análise.

A organização modular é essencial para que a evolução possa acontecer, por criar uma certa independência entre os caracteres. Em última instância, a própria existência de caracteres identificáveis é uma evidência da importância da organização do organismo em módulos relativamente independentes. Wagner e Altenberg (1996) foram pioneiros no entendimento da modularidade como uma adaptação para permitir que os organismos tivessem a capacidade de responder à seleção de forma eficiente, e nesse contexto apresentam a modularidade como uma solução a uma série de problemas aos quais os organismos estão sujeitos ao interagir com os processos evolutivos, como a necessidade de evolução coordenada de caracteres funcionalmente ligados e a robustez a mudanças em outras partes do organismo.

1.3.1 Evolução da covariação genética

A evolução da covariação genética é o tema central desta tese, e para pensar no padrão de covariação como uma adaptação é preciso entender como a variação genética entre indivíduos contribui para variação do próprio padrão de covariação, para que o padrão de variação possa evoluir. Por um lado, é claro que a variância aditiva pode mudar ao longo do tempo pela simples mudança na frequência de alelos aditivos. Por exemplo, uma população que perde variação genética por endocruzamento pode perder toda a variação genética se novos alelos não forem introduzidos por mutação, e isso obviamente mudaria o padrão de covariação genética, pois não haveria variação. Na prática isso é extremamente raro na natureza, e a grande maioria dos caracteres apresentam variação genética suficiente para responder à seleção. Além disso, empiricamente podemos observar certa constância nos padrões de covariação genética em

uma escala de tempo evolutivo, mesmo que existam flutuações de curto prazo. De alguma forma, não só a variação genética é mantida em níveis consideráveis, mas a estrutura dessa variação parece ser adaptativa e relativamente estável, com padrões modulares associados ao desempenho de funções. A maneira como essa variação é mantida e como os padrões são estabelecidos e mantidos é um problema fundamental em biologia evolutiva.

A manutenção da variação genética aditiva foi um tema absolutamente central em biologia evolutiva durante os últimos 30 anos, e continua sendo. Não vamos abordar este tema diretamente, mas cabe fazer um breve comentário sobre os processos que mantêm a variação genética. A genética quantitativa assume que existe variação aditiva suficiente para responder à seleção, e no geral essa hipótese é bastante razoável. A imensa maioria dos caracteres e combinações de caracteres apresenta variação genética apreciável, mesmo em sistemas multivariados e sujeitos à seleção natural. Um exemplo interessante de teste empírico da capacidade de um sistema multivariado de responder à seleção em qualquer direção foi feito por Hine et al. (2014), e os resultados foram típicos: todas as direções do espaço fenotípico têm variação suficiente para responder à seleção. Vários processos contribuem para essa manutenção de variação, como migração, sobre-dominância na aptidão, efeitos antagonistas de alelos na aptidão ao longo da vida, variação espacial na superfície de seleção. Todos esses processos são importantes, mas o mais importante para a manutenção da variação, e que, em última instância, dá origem a toda variação, é a mutação. De alguma maneira, o influxo de novas variantes por mutação equilibra a remoção de variação por deriva e seleção. A forma exata desse equilíbrio mutação-seleção-deriva não é bem entendida, e diferentes aproximações levam a expectativas diferentes variação aditiva no equilíbrio. Existem duas aproximações comuns para o estudo analítico do equilíbrio mutação-seleção-deriva, e essas aproximações diferem principalmente no seu tratamento do comportamento de novas mutações. Na aproximação Gaussiana, ou aproximação do contínuo de alelos, utilizada por Kimura (1965) e Lande (1975), novas mutações são representadas por pequenas mudanças nos efeitos anteriores dos alelos, e portanto o efeito do novo alelo depende do efeito antigo. Nessa aproximação, a variância

esperada no equilíbrio se deve a um grande número de alelos segregando em cada locus, e portanto cada locus contribui muito para a variância aditiva. Como a variância é mantida por muitos alelos, Turelli (1984) apontou que essa aproximação depende de uma taxa de mutação muito alta para ser realista. No outro extremo, temos a aproximação de *House-of-cards* defendida por Turelli (1984), na qual novas mutações geram alelos com efeitos completamente independentes dos efeitos anteriores⁸. Nessa aproximação, a maior parte da variação se deve principalmente a alelos raros, e isso implica que essa aproximação é aplicável mesmo com taxas mutacionais mais baixas. Apesar disso, na aproximação de *House-of-cards*, o número de loci necessários para explicar a variação encontrada em populações naturais é bastante alto, e nosso entendimento da arquitetura genética de caracteres complexos ainda não permite dizer se essa aproximação é mesmo realista. Então, das duas aproximações apresentadas para explicar a variação encontrada em populações naturais como um equilíbrio entre mutação e seleção, uma depende de taxas mutacionais elevadas e a outra, de um número de loci elevado controlando cada caráter. A inclusão de complicações como epistasia e pleiotropia também não fornece um quadro claro do equilíbrio entre mutação e seleção (Turelli 1985), apesar de interações epistáticas parecerem ser uma fonte importante e ainda mal documentada de variação aditiva. Em resumo, dadas as taxas mutacionais estimadas, a maioria dos modelos prevê menos variação aditiva do que nós observamos. Rice (2004) comenta como essa situação é peculiar: o problema da teoria evolutiva é justamente que a seleção pode ser mais efetiva que o previsto pelos modelos.

A segunda questão, referente à origem e manutenção do padrão de covariância genética, é mais recente, e estamos começando a entender como os efeitos genéticos e o processo de desenvolvimento permitem a evolução nos padrões de covariância. Em particular, interações epistáticas têm se mostrado fundamentais para a geração de variação nos padrões de covariância, fornecendo um mecanismo para a alteração de padrões pleiotrópicos (para uma revisão recente veja Pavlicev e Cheverud (2015)). A abundância dessa variação epistática garante

⁸Daí o nome da aproximação: cada nova mutação muda completamente a distribuição de alelos, destruindo a distribuição anterior como um castelo de cartas.

que os padrões podem efetivamente evoluir em populações naturais. Além disso, a variação epistática pode também afetar o padrão de correlação mutacional de um gene. O padrão de correlação entre os efeitos mutacionais afeta o padrão de covariância, pois novas mutações que afetem mais de um caractere têm o potencial de criar ou remover correlações entre caracteres. Na presença de seleção estabilizadora, existe uma expectativa teórica de que os padrões de correlação mutacional, genético e seletivo se alinhem, mantendo o padrão de covariação estável (Cheverud 1984). Em populações naturais, foi documentada tanto a manutenção de padrões de covariação em escala macro evolutiva (Marroig e Cheverud 2001), quanto mudanças adaptativas nos padrões de covariação (Young e Hallgrímsson 2005). Quais os mecanismos seletivos, genéticos, mutacionais e de desenvolvimento envolvidos na alteração ou manutenção dos padrões de efeitos pleiotrópicos e de covariação ainda é uma questão em aberto em biologia evolutiva.

1.4 Esquema da Tese

Nesta tese, abordamos a questão da evolução e manutenção dos padrões de covariação utilizando diversas populações experimentais submetidas a regimes de seleção diferentes, simulações computacionais e técnicas estatísticas modernas para ligar os efeitos genéticos à covariação e entender o efeito da seleção na covariação genética e nos padrões pleiotrópicos. A tese é apresentada em cinco capítulos relativamente independentes mas ligados pelo tema da evolução da covariação genética. Com exceção do Capítulo 5, que depende do Capítulo 4 em alguns passos técnicos, todos podem ser lidos independentemente.

O Capítulo 2 (*How does modularity in the genotype-phenotype map interact with development and evolution?*) apresenta uma revisão básica do conceito de modularidade e discute as causas e consequências da organização modular da arquitetura genética. Este capítulo pode ser visto como uma segunda introdução, mais técnica e motivadora dos capítulos seguintes.

O Capítulo 3 (*The Evolution of Phenotypic Integration: How directional selection reshapes*

covariation in mice) aborda o problema da evolução da intensidade da correlação fenotípica e genética após um episódio de seleção direcional. Utilizando camundongos provenientes de um experimento de seleção artificial para alteração do peso corporal, nós medimos o padrão e a intensidade de correlação em caracteres cranianos em duas linhagens selecionadas para aumento de tamanho, duas linhagens selecionadas para diminuição de tamanho, e uma linhagem controle. Nossos resultados mostram que o padrão de covariação muda com a seleção direcional, aumentando a proporção de variação alinhada com a seleção direcional, e portanto aumentando também a evolvabilidade relativa na direção de seleção. Este capítulo foi escrito em colaboração e tem Anna Penna como co-primeira autora. Os dados foram derivados de um projeto de iniciação científica realizado no Laboratório de Evolução de Mamíferos do IB-USP, e os animais utilizados no projeto foram cedidos pela professora María Inés Oyarzabal da Universidade de Nacional de Rosário, Argentina. O capítulo foi publicado na revista *Evolution* (doi:10.1111/evo.13304).

O Capítulo 4 (*Genomic Perspective On Multivariate Variation, Pleiotropy, And Evolution*) utiliza uma população de camundongos provenientes de duas linhagens puras para estudar como o padrão de efeitos genéticos se relaciona com o padrão de covariação de um conjunto de caracteres da curva de crescimento. As linhagens puras são derivadas de dois experimentos independentes de seleção no peso corporal, um para aumento de peso e um para diminuição de peso. Utilizando técnicas de mapeamento genético e a teoria de genética quantitativa, nós pudemos particionar o efeito dos componentes aditivos e de dominância na covariação genética, e mostramos que o padrão de covariação genética é o resultado da combinação da influência da seleção e de restrições internas que moldam os efeitos pleiotrópicos. Além disso, também compararamos duas técnicas de mapeamento genético na sua capacidade de inferir o fenótipo das linhagens puras utilizando somente os efeitos genéticos estimados na população intercruzada.

O Capítulo 5 (*Genetic Architecture and the Evolution of Variational Modularity*) aplica os mesmos métodos desenvolvidos no capítulo anterior para relacionar efeitos genéticos com a

covariação em uma linhagem intercruzada de camundongos formada por seis linhagens puras advindas de um experimento de seleção direcional para a alteração da curva de crescimento. Nesse experimento de seleção artificial, foi observada uma mudança marcante no padrão de covariação genética após a seleção, com a eliminação de uma correlação positiva entre as fases iniciais e finais do crescimento. Utilizando a população intercruzada, nós exploramos a base genética do novo padrão de covariação e relacionamos esse padrão e os efeitos genéticos com a história evolutiva das linhagens selecionadas. Além disso, utilizamos simulações baseadas em indivíduos para estabelecer expectativas sobre a distribuição de efeitos pleiotrópicos sob diferentes tipos de seleção.

O Capítulo 6 (*Modularity: Genes, Development, and Evolution*) é uma revisão abordando tanto as bases genéticas do padrão de covariação quanto sua evolução e as consequências macro evolutivas da covariação genética. Sendo uma revisão bastante geral, o capítulo funciona como uma introdução relativamente completa aos temas gerais da tese e como uma discussão das consequências dos temas abordados nos capítulos experimentais anteriores, mais específicos. Este capítulo foi escrito em colaboração e tem Arthur Porto como co-primeiro autor. O capítulo foi publicado na revista *Annual Review of Ecology, Evolution, and Systematics* (doi:10.1146/annurev-ecolsys-121415-032409).

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Chapter 2

How does modularity in the genotype-phenotype map interact with development and evolution?

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Abstract

Traits do not evolve independently, as genetic and developmental associations affect the variation that is expressed in populations and that is available for evolutionary change. In this chapter, we explore the causes and consequences of structured variation, introducing the concept of modularity, exploring some possible causes for modular organization in different levels, and finally, discuss how the introduction of new variation can evolve

Keywords: G-matrix; QTL mapping; genome prediction; genetic architecture

2.1 Evolution and variation

“Hence if man goes on selecting, and thus augmenting, any peculiarity, he will almost certainly modify unintentionally other parts of the structure, owing to the mysterious laws of correlation.” – Darwin (1872)

Evolution proceeds by many different processes, all of which depend on the variation present in natural populations. The probability of fixation or loss of a neutral variant due to drift depends on its frequency in a population. The increase or decrease via natural selection of the frequency of an allele that has an effect on fitness depends on the standing variation in that locus. Therefore, the fate of a new variant depends on the population in which the new variant appears, whether it is neutral or not. Advantageous variants that are quite frequent may be lost in small populations, while even the smallest advantage in fitness can guarantee that a rare variant will be fixed in very large populations. In an analogous way, the change in the distribution of a phenotype in a population depends on its standing variation, and the details of this variation can profoundly alter the evolutionary process. For example, consider a hypothetical selection regime that operates as to increase the length of the left arm of the individuals in a population. Individuals that have a long left arm leave more offspring, and if the trait is heritable, the offspring of these individuals have themselves longer left arms. So, the mean length of the left arm increases due to natural selection. But the right arm is seldom very different from the left arm, and so these long-left-armed individuals also have long right arms, and the mean of the right arm also changes between generations, due to a process we now recognize as correlated response to selection (Lande and Arnold 1983). This indirect correlated response happens because left and right arms are genetically correlated, and when the genes for long-left-arm are passed on to the next generation in large numbers, they are also genes for long-right-arm, resulting in an evolutionary change in a structure that was not under selection. This example illustrates that, while individuals are composed of multiple parts, these parts are not independent, and how they are related in a population can indeed

alter evolutionary outcomes. This is a problem familiar to all animal breeders throughout history (Hazel 1943), as attempts to selectively breed individuals in order to improve some aspect of the populations invariably leads to changes to other aspects. This problem suggests that a more complete understanding of diversification and evolution must somehow include a model for the evolution of the relations between traits.

Phenotypes are always a complicated affair. Even the simplest of biological structures is composed of several different parts and must be created from scratch in each individual, requiring several thousand proteins and molecules to interact in some way. All the different traits in an individual must be formed through development, and the blueprints for this process is encoded in the genome. So, if a phenotype is formed by development, then it must follow that all the variation in these phenotypes in a population must also be created, or at least mediated, by development. There are several sources of variation in biological populations. Some proportion of the variation in a population will be due to differences in the genetic makeup of individuals, while some other part will be due to variation inherent to the development process, some part will be due to environmental differences between individuals, and so on. Because all these sources of variation must be channeled through development, the structure of development imposes constraints in the variation that is ultimately expressed in a population. If the left and right arm share, to some degree, some developmental pathways, variation in these pathways will lead to correlated variation in both arms. The path between genetic variation and phenotypic variation is crucial and can be expressed in the genetic architecture.

2.2 Genetic architecture and the genotype-phenotype map

Genetic architecture is the structure of the relation between genotype and phenotype (Hansen 2006). Genetic variation acts through some sort of developmental process to produce variation in the phenotype. But not all genetic variation will affect the full phenotype of the individual. Some parts of the genome only affect very specific phenotypes, while others will have gener-

alized effects in the whole organism. Genetic architecture defines this relation and can have important consequences to the evolutionary process, while at the same time being modified by it.

One way of understanding genetic architecture is by constructing genotype-phenotype maps (GP maps), which are mappings between genetic sequences and phenotypes. Here, phenotypes can refer to a broad range of traits, from exceedingly simple to the mind-bogglingly complex. Perhaps the simplest possible example of a GP map that is still relevant in biology is the secondary structure of an RNA molecule. For a given RNA molecule sequence (genotype), the very simple development of folding the molecule results in a particular shape (phenotype) (Ancel and Fontana 2000). This shape is uniquely determined by the sequence, but different parts of the sequence interact in complicated ways to generate the full final shape. Because of the richness of the relation between sequence and shape, this simple system has been extensively used as a model of evolution (Stadler and Stadler 2006), and indeed presents several properties that we aim to understand in more complex phenotypes, such as modularity and robustness. We can also think of the genetic architecture and GP map of more complex traits, such as behavior, gene expression, skeletal structures, growth, body shape, body composition and so on. Evolution of these complex traits, composed of several interacting parts, is profoundly influenced by genetic architecture.

We often rely on mathematical models to explore the consequences of assumptions on the structure of the GP map. One of the earliest models of complex phenotypic change, Fisher's geometric model (Fisher 1930), already presented formidable consequences to evolution (Orr 2000). In this model, Fisher assumed a completely pleiotropic genetic architecture. Pleiotropy is the situation where one gene affects more than one unrelated trait, and in Fisher's model, all genes affected all traits. An individual is represented as a point in some high dimensional continuous phenotype space, with each dimension representing some trait in the individual, and fitness is given by a selective surface with a single optimum. Mutation is represented by a shift in all traits, and this can be interpreted as a vector sum between the initial position,

and a small random vector representing mutation. This mode of mutation implies complete pleiotropy, as every mutation can potentially affect every trait. Furthermore, this geometric interpretation of mutation gives the model its name. If the individual is not at the phenotypic optimum, a mutation might increase or decrease fitness. If the mutation increases fitness, it can be fixed via natural selection and lead to adaptation, with probability proportional to the increase in fitness. Under this model, Kimura (1983), and later Orr (2000), showed that the rate of adaptation decreases with the number of traits, an effect called “cost of complexity”, as more complex organisms would be slower to adapt. This cost appears because, under complete pleiotropy, only a small proportion of random mutations would alter all the traits of an organism in a beneficial way, and most mutations will move the individual away from the optimum. This result was shown to be fairly robust to some possible mitigating assumptions regarding the genetic architecture (Welch et al. 2003), and so posed a difficult mismatch between observation and theory, as complex organisms composed of many traits exist and seem to have no problem adapting to many environments, sometimes with remarkable speed (Kinnison and Hendry 2001).

This paradox only became tractable in light of explicit tests of the assumptions of the geometric model, namely the pattern of pleiotropy in the GP map and its consequences to mutation. In the last few decades, we have begun to experimentally explore the genetic architecture of complex traits using molecular mapping techniques, which allows us to relate genetic variation to phenotypic variation (Mackay 2001). Quantitative trait loci (QTL) studies and genome wide association studies (GWAS) have allowed us to investigate which variants are related to disease, to improve our agricultural efficiency, to develop optimal breeding strategies, and to further our understanding of the evolutionary process by directly assessing genetic architecture. Using QTL mapping, Wagner and collaborators (2008) investigated the assumptions the geometric model made with regards to the GP map and showed that the assumptions of the geometric model are not reasonable. This incongruence between data and Fisher’s early model of genetic architecture can be summarized by two key points. First,

pleiotropy is not global, and the vast majority of loci affect only a small number of traits. Second, the pleiotropic effect of loci onto a trait does not decrease with the loci's level of pleiotropy. In other words, there is a non-trivial scaling of genetic effects with the degree of pleiotropy. It turns out these details matter a great deal here, and these properties of the genetic architecture of complex traits we were only recently able to quantify experimentally are, therefore, fundamental for the evolvability of complex organisms (Wagner and Zhang 2011). We may ask how these evolvable genetic architectures came to be.

2.3 Traits and modules

“It [adaptation of traits] can only be workable if both the selection between character states and reproductive fitness have two characteristics: continuity and quasi-independence. Continuity means that small changes in a characteristic must result in only small changes in ecological relations; a very slight change in fin shape cannot cause a dramatic change in sexual recognition or make the organism suddenly attractive to new predators. Quasi-independence means that there is a great variety of alternative paths by which a given characteristic may change, so that some of them will allow selection to act on the characteristic without altering other characteristics of the organism in a countervailing fashion; pleiotropic and allometric relations must be changeable. Continuity and quasi-independence are the most fundamental characteristics of the evolutionary process. Without them organisms as we know them could not exist because adaptive evolution would have been impossible.”

— Lewontin (1979)

“But what are the structural features that make stepwise improvement possible? The key feature is that, on average, further improvements in one part of the system must not compromise past achievements (...)” — Wagner and Altenberg (1996)

The very existence of (semi)individualized traits depends on one ubiquitous aspect of

evolved genetic architecture and genotype-phenotype maps: modularity. Modularity can be defined very generally as a property of a system whose parts are assembled into groups which are tightly associated, while maintaining a relative independence between groups. In the context of biological organisms, modularity appears at several levels of organization. Traits are, in a sense, modules, recognizable units with relative independence, and indeed Richard Lewontin went so far as to postulate that this subdivision is fundamental to adaptation (Lewontin 1979). The cost-of-complexity paradox illustrates this nicely: without a genetic architecture that provides some level of independence between traits, adaptation can not occur. It is not surprising then that these identifiable units we recognize as traits are modified during evolution without severely affecting the rest of the organism, and, accordingly, their independence is reflected in their genetic architecture. Hansen (2003) points out that a modular genetic architecture is not the only way to achieve independent traits, but the observed modular organization at several levels of organization, from gene expression to morphology, suggests a deep underlying principle of organisms (Wagner et al. 2007).

Modularity occurs at several levels, and traits are organized into larger modules, which may, for example, perform a given function or form a structure. Olson and Miller (1958) founded a research project based on the holistic investigation of organisms and their organization into interconnected groups and in their interrelations form the whole individual. Their seminal book championed the idea of morphological integration, which captures the varying degree of interdependence between traits must pose in order to come together into functional units and that can then perform the functions that are required of them. Olson and Miller pointed out that we can identify these groups of traits by their correlations, as traits in a functional module should covary together, as a consequence of their mutual requirements for the performance of a function. The importance of these modules to evolution was elegantly posited by Wagner and Altenberg (1996), who brought the idea of a modular architecture as a central concept of biological organization. These modules of correlated morphological traits could then change with relative independence during evolution, while the genetic correlations

within modules facilitate a coordinated response to selection, maintaining their function if one of the elements within a module were to be altered (Cheverud 1982, 1984).

The advent of QTL mapping also allowed us to investigate how the relative independence between these sets of traits related to the genetic architecture. Studies in several levels of biological organization show that the genetic architecture underlying these modules is also modular, as the pleiotropic effects of genes are more often restricted to traits within these groups. This is true of gene expression (Hartwell et al. 1999; Segal et al. 2003) all the way up to morphological traits (Mezey et al. 2000). Modularity is also expressed in development, as the several processes involved in the formation of a given trait will also be relatively separate from one another and conceptually different. To see this, we may return to the example of the left and right arm. Both develop separately, and so in some sense, each is formed by a separate developmental module, but both share a great deal of genetic information and tend to evolve together, and so are the same evolutionary module. However, these relations are not static, as evolutionary and developmental modules can be created or destroyed during evolution. One familiar example of this kind of modular reorganization is the case of the association between upper and lower limbs in humans, which became less associated as a result of changes in our mode of locomotion (Young et al. 2010). Changes in the pattern of correlations between phenotypic traits suggest the underlying modular GP map responsible for the genetic associations we observe in populations can be altered by evolution. This realization has important consequences, as we establish a feedback between selection and associations. We now turn to the first part of this feedback.

2.4 Evolution of modular GP-maps

Advances in QTL mapping have allowed us to probe the genetic architecture and describe the mechanistic basis for the evolution of genetic architecture, and the origin of the genetic variation that can allow changes in the GP map. Cheverud and colleagues have shown that

gene interactions are a major source of variation in pleiotropic patterns (Pavlicev et al. 2008; Wolf et al. 2005). Epistasis, gene effects that depend on the interaction between different loci, greatly enhances the variational possibilities in natural populations by changing which loci affects which traits. More importantly for our discussion, variation in pleiotropic relations provide the necessary ingredient for us to understand the evolution of modularity (Wagner and Altenberg 1996). The link between function and modules of phenotypic traits suggest that modular organization is an adaptation, and so we focus on selective explanations for the modular organization of genetic architecture. However, plausible neutral mechanisms for the emergence of modularity in some organizational levels have been proposed in the literature (Lynch 2007; Wagner et al. 2007). Furthermore, since modularity is so general and occurs at different levels of biological organization, it is hard to imagine this property evolved by a single mechanism at all scales, and therefore each kind of association might require different explanations. Perhaps the ubiquity of modularity reflect these different roads that lead to it. Therefore, how modularity evolves remains an open question, and because all of these modular architectures at different levels are already established in nature, the work on the possible causes for its evolution relies heavily in mathematical and computational models.

One of the important problems I have emphasized that is solved by modularity is the need for robustness, in the sense that changes in one part of the organism does not interfere with the others. Ancel and Fontana (2000) used a model for the secondary structure of RNA molecules to study the origins of modularity, defined as the independence between different parts of the RNA molecule in the process of melting under increasing temperature. In a modular molecule sequence, secondary structure is lost in the different parts of the molecule (modules) independently, while in a non-modular sequence, the whole molecule continually changes its configuration during melting. In their simulations, stabilizing selection was applied to a population of evolving sequences, based on their secondary structures. This selection for robustness had a number of consequences in the GP map of the sequences. Selected sequences were more robust to mutations, showed less phenotypic variation at intermediary

temperatures, there was a convergence of phenotypic and genetic variation, and the selected sequences also became more modular (i. e., conformations in different parts of the molecule become more independent). This suggests that direct selection for robustness can lead to the evolution of modularity, but the increase in evolvability due to modularity is not present in this simplified system, so the analogy breaks down somewhat. In any event, it is quite possible that selection for robustness is a driver of the evolution of modularity.

Selection for evolvability has also been proposed as a possible cause of modularity. In quantitative genetics, evolvability is defined as the available variation for the response to selection, and Pavlicev et al. (2011) proposed a model based on the existence of genetic variation for the association between two traits. This variation was expressed in the form of an additive Mendelian polymorphism for the correlation between two traits in a population. Homozygous individuals for one allele contribute to high correlation between the traits, while homozygotes for the other allele show no correlation, and the heterozygotes show intermediary correlations. Selection was modeled deterministically using the response to selection equation from quantitative genetics theory. Under this model, selection for coordinated evolution of the two traits (simultaneous increase or decrease in the value of the traits) leads to the fixation of the allele encoding high correlation, and corridor selection, when one trait is held constant and the other traits is selected for increase, leads to the fixation of the allele encoding low correlation. In these two scenarios, the allele that provides the highest amount of variation in the direction of selection is fixed, and so selection increases evolvability by either integrating or modularizing trait variation. We observed a similar effect of directional selection in a fully mechanistic model, where pleiotropy and gene effects were allowed to change via mutation in a large population of simulated individuals. Using this model, we were able to show in Melo and Marroig (2015) that stabilizing selection and drift are not viable candidates for the emergence of modularity in complex phenotypes composed of many traits. Stabilizing selection was theoretically a possible driver of modularity (Cheverud 1984; Lande 1980), and has been shown to be effective in a small number of traits (Jones et al. 2007, 2014), but the structure

of high dimensional variation prevents stabilizing selection from being efficient for multiple traits. This difficulty appears because stabilizing selection is very efficient at increasing within-module correlations, but not efficient at reducing between- module correlations, so modules can't form. We looked at the effect of directional selection on the covariance structure and the pattern of pleiotropic relations, and we see that directional selection is a powerful driver of modularity. Traits that are selected in the same direction in the simulations rapidly become more associated than traits that are selected in different directions. Also, we show that corridor selection can create complex patterns of correlations, as traits under directional selection become more associated within themselves, while traits under stabilizing selection maintain an intermediate level of correlation, and the correlation between these two groups is reduced. In all simulation, the changes in the correlation structure are due to selective changes in the GP map, in which pleiotropic relations are altered by selection, increasing evolvability.

Moving to some non-morphological traits, selection for more than one function has also been shown to promote modularity in gene regulation networks, while single-objective networks were more integrated (Espinosa-Soto and Wagner 2010). This is somewhat analogous to the continuous traits case we discussed above, where different parts of the system become adapted to one function. These modular regulation networks are also more stable and robust. Interestingly, when working with neural networks, selection for multiple objectives was not sufficient for creating modules in work done by Clune et al. (2012). In their simulations, in addition to the selection for multiple outputs, the neural networks only became modular with the addition of a cost for connections between nodes of the neural network. While only suggestive, this provides a possible explanation to why modularity and not other pleiotropic organization that provide evolvability [see Pavlicev and Hansen (2011) for examples] are more common in nature: there could be a cost to maintaining high levels of pleiotropy, even if not in the form of low evolvability.

All the models we've seen so far treat development as a black box that does not influence modularity, which is clearly a rather strong simplification. In an attempt to include the

complications of development, Watson et al. (2014) use an ingenious strong selection weak mutation model that allow them to include explicit developmental interactions to the GP map. In this model, both the initial (embryonic) traits and the interactions between these traits in all phases of development are under genetic control. At each step of development new interactions add complexity to the final adult phenotype, and this adult phenotype is exposed to selection regimes that can change every few thousands of generations. Traits in this model tend to become more associated throughout development when they are selected in the same direction in all selection regimes, and become independent when they are selected in different directions. Also, selection for different independent modules can lead to developmental interactions that allow composition of these modules to form novel morphologies that were not the initial selected states, an emergent form of complex organization. We now turn to these emergent properties of modularity that can profoundly facilitate adaptation.

2.5 Modular variability

“Evolvability is the genome’s ability to produce adaptive variants when acted upon by the genetic system. This is not to say that the variants need to be ‘directed’ (Foster and Cairns 1992) for there to be evolvability, but rather, that they cannot be entirely ‘misdirected,’ that there must be some small chance of a variant being adaptive. The situation is analogous to obtaining a verse of Shakespeare from monkeys banging away on typewriters. Typewriters make this far more likely than if the monkeys had pencil and paper. The type-writers at least constrain them to produce strings of letters. Similarly, the genotype-phenotype map constrains the directions of phenotypic change resulting from genetic variation.” – Wagner and Altenberg (1996)

Perhaps the most interesting consequence of the modular structure of the GP map and development is the effect this organization has on variability. Günter Wagner has often drawn the distinction between variation and variability (Wagner and Altenberg 1996). For our pur-

poses, *variation* refers to the expressed differences between individuals in a given population: how different are they, or how differences between individuals are correlated. Using variation we might predict how a population evolves under drift or natural selection, or make inferences regarding variational modules. *Variability*, on the other hand, refers to the ability of the population to generate variation. Wagner likens variability of an organism to the solubility of a substance. Solubility does not refer to the physical state of being in solution, but instead to a property that a given substance has that defines how it behaves when in solution. Likewise, a population of genetically identical individuals has no genetic variation, but still has variability, defined by its mutational properties. (For example, new mutations could have correlated effects on many traits due to shared development and genetic architecture.) Variation present in populations that is available for selection must ultimately come from mutation. We are often told that mutation is random, but this is a rather strong simplification. In what sense are mutations random? Dan Graur [Graur (2015), pp. 34] points out that mutations are not random with respect to genome position or mutation type, and that mutational effect on fitness are species specific, gender specific, developmental stage specific, and several other non-random conditions. The only way in which mutations are random is in that the probability of a given mutation is the same regardless of whether it is advantageous, neutral, or deleterious in the individual in which it appears (Luria and Delbrück 1943). The key point is that new mutation can be structured by variability, and so new variation can also be structured. In quantitative traits, we can describe and quantify variability by using the mutational matrix, the covariance matrix of mutational effects. This can be done experimentally using mutation accumulation lines, measuring the correlation between phenotypic changes that appear in these lines due to mutation. We expect that, under some general conditions and given enough time, the genetic variation in a population come to mirror the mutational matrix (Cheverud 1984; Jones et al. 2007; Lande 1980). The form of the mutational matrix, and of variability in general, depends on the GP map and on development, as traits that share pleiotropic genes or developmental pathways will be jointly altered by mutations. So, all the results we have seen on selection

altering GP maps have consequences to variability and to the introduction of new variation in natural populations.

Models for the evolution of the mutational matrix in quantitative traits reveal the possibility for interesting dynamics. Jones et al. (2014) used an individual based model with epistatic interaction to study the evolution of the mutational matrix. Epistasis is important because it opens the door for complex interactions, and can lead to variation in mutational correlations. Under their model, the mutational matrix of two quantitative traits evolves to match the selection surface matrix, and so new mutations are biased by past selection. Consequently, variation that is introduced by mutation tends to conform to the past selective surface, and if this surface is stable, new mutations have a lower probability of being deleterious. This kind of reorganization of variability also appears under directional selection in the model from Pavlicev et al. (2011) and in Draghi and Wagner (2008), which uses a different scheme for the evolution of pleiotropic relations and trait associations.

While these mathematical and computational results are remarkable, they are difficult to explore experimentally. Epistasis and allele interactions have been shown to contribute significantly to the phenotypic covariation in complex traits (Cheverud et al. 2004; Huang et al. 2012; Pavlicev et al. 2008; Wolf et al. 2005, 2006), but we still lack a deep understanding on how this variation is explored by natural selection and evolution. However, recently studies in natural populations and artificial selection have begun to uncover the effects of selection on covariation. Working with morphological skull traits, Assis et al. (2016) (in natural populations) and Penna et al. (2017) (in artificial selection experiments) have shown that variation can indeed be reorganized in the direction of selection, increasing potential future evolvability, the same kind of effect observed in simulations in Pavlicev et al. (2011) and Melo and Marroig (2015). Conversely, several studies have documented the opposite effect, in which directional selection acts in a more traditional manner in multivariate traits, eroding the genetic variance in the direction of selection (Walsh and Blows 2009). Careau et al. (2015) carefully documented this effect in behavioral traits in mice using a large selection

experiment, in which response to selection plateaued after several generations of selection. These differences might be explained by differences in the genetic architecture of these two different types of traits, but more detailed studies are certainly needed.

2.6 Phenotypic space and concluding remarks

This remarkable feedback between selection, variation and variability suggests a deeper consequence of the structure of the GP map and phenotypes. Most of our understanding and descriptions of phenotypes assume that the space in which phenotypes exist is continuous, Euclidean, and that we can measure how close two phenotypes are using a natural distance measure. In this framework, we rely on carefully chosen adaptive landscapes to explain why some portion of the phenotypic space are not explored, and to account for the emergence of modularity. If not for selection, this framework implicitly places no limitations on the possible phenotypes of organisms. Stadler et al. (2001) provide a different perspective, wherein phenotypic space is such that simple Euclidean distances do not make sense (like the surface of Earth at large scales), and phenotypes are not restricted only by selection, but also by development and genetic architecture. In this space, distances depend on genetic proximity and the GP map, thus limiting the set of paths that the mean phenotype of a population can take. In this view, modularity and robustness and several other unexplained complexities in phenotypic evolution are a reflection of the underlying metric imposed by the GP map. A simpler and less encompassing version of this idea was already present in the quantitative genetics literature. For example, Lande (1979) explicitly stated that a population's distance to an adaptive peaks should be measured in genetic variance distance, not morphological distance, and see Steppan et al. (2002) and Melo et al. (2016) for an exploration of the macroevolutionary consequences of this fact.

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Chapter 3

The Evolution of Phenotypic Integration: How directional selection reshapes covariation in mice

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Abstract

Variation is the basis for evolution, and understanding how variation can evolve is a central question in biology. In complex phenotypes, covariation plays an even more important role, as genetic associations between traits can bias and alter evolutionary change. Covariation can be shaped by complex interactions between loci, and this genetic architecture can also change during evolution. In this article, we analyzed mouse lines experimentally selected for changes in size to address the question of how multivariate covariation changes under directional selection, as well as to identify the consequences of these changes to evolution. Selected lines showed a clear restructuring of covariation in their cranium and, instead of depleting their size variation, these lines increased their magnitude of integration and the proportion of variation associated with the direction of selection. This result is compatible with recent theoretical works on the evolution of covariation that take the complexities of genetic architecture into account. This result also contradicts the traditional view of the effects of selection on available covariation and suggests a much more complex view of how populations respond to selection.

Keywords: macroevolution, genotype–phenotype map, G-matrix, adaptive landscape, morphological integration

3.1 Introduction

Evolutionary change can only occur in the presence of variation, and when dealing with complex multivariate phenotypes (consisting of multiple traits) the patterns and magnitude of genetic covariation between traits can radically influence the course of evolution (Felsenstein 1988; Lande 1979). The standing genetic covariation of a given population depends on its evolutionary history, and can be altered by selection, drift, mutation and recombination (Jones et al. 2004, 2014; Turelli and Barton 1994). These changes in covariation, in turn, can alter how a population responds to further selection or other evolutionary processes. So, if we are to understand how populations evolve and how the current phenotypic diversity observed in nature came to be, then the question of how genetic variation changes under various evolutionary processes becomes central to biology (Mitchell-Olds et al. 2007).

How a single trait responds to directional selection is a well studied problem (Falconer and Mackay 1996). In general, we expect the response to selection to gradually erode genetic variation, as the many loci influencing a given trait go to fixation and, in the absence of mutation, preclude further evolutionary change (Bulmer 1971). If mutation is present and of sufficient magnitude, the variation removed by selection can be replenished and the rate of evolutionary change remains constant, at least for a time.

A theory on how directional selection and covariation interact to produce the response to selection on multiple traits was proposed by Lande (1979). This author related the standing genetic covariation, represented by the additive genetic covariance matrix (G), to the selection gradient (β), a vector of selection coefficients acting independently on each trait, to predict the response to selection ($\Delta\bar{z}$). The Lande equation ($\Delta\bar{z} = G\beta$) predicts a response that is dependent on covariation, which means that selection can even lead to changes in traits that were not directly under selection (Cheverud 1984). Since more traits are presumably affected by more loci, in the multivariate case genetic architecture can become quite complicated, and consequently the erosion of genetic variation by selection is much more complex (Pavlicev

et al. 2008; Wagner and Zhang 2011; Wolf et al. 2000). This conflict between selection and the maintenance of genetic variation in multiple traits remains a fundamental and puzzling problem in evolutionary biology (Walsh and Blows 2009).

One way to investigate the effects of selection on covariation is to use artificial selection experiments. Wilkinson et al. (1990) used experimental *Drosophila* populations selected for body size and found significant differences in correlation patterns between the different directions of selection, and even changes in the sign of some genetic correlations. Notwithstanding, analyzing the same dataset, Shaw et al. (1995) showed that the differences found by Wilkinson et al. (1990) are compatible with drift. Bryant et al. (1986) and Whitlock et al. (2002) also used *Drosophila* to show that both an increase and a decrease of genetic variation is possible under drift, probably due to genetic interactions like dominance and epistasis (Cheverud and Routman 1995). Taken together, these results present a conflicting picture on how covariation evolves, and on the potential evolutionary consequences of these changes.

On a macroevolutionary scale, retrospective studies have attempted to quantify the interplay of genetic constraints and phenotypic divergence. Pitchers et al. (2014) found no consistent pattern on how genetic covariation and intensities of selection affect the magnitude of evolutionary response. However, they did not take the multivariate aspect of the phenotype space under consideration and, since the orientation of selection alters the available variation for response, this can explain the lack of a clear pattern. As for the general pattern of bivariate correlations, Roff and Fairbairn (2012) surveyed estimates of selection gradients and correlations to test the hypothesis that high correlation between co-selected traits are advantageous, and found that traits that are selected in the same direction indeed tend to be more correlated than average. A well studied case is the mammalian cranium, where covariation patterns tend to be stable (Porto et al. 2009), and phenotypic divergence is frequently size related and aligned with the main axis of within-population covariation, the genetic line of least resistance (Marroig and Cheverud 2010; Marroig et al. 2012; Porto et al. 2013; Schluter 1996). If this alignment of divergence and variation is a consequence of genetic

constraints limiting the response to selection, or a case of selection altering covariation, or both, is still an open question.

Recently, several attempts have been made to directly investigate the behavior of the G-matrix under directional selection. Careau et al. (2015) used a large population of mice to select for changes in a multivariate behavior trait, and showed that selection reduced the available variation for adaptive response, significantly reducing the rate of adaptation after a few generations and reducing the amount of variation in the direction of selection. This is consistent with a traditional model of selection depleting variation extended to a multivariate context: even when variation is present in all traits in a complex system, combinations of traits may lack additive variation to respond to selection (Hine et al. 2011). On the other hand, Assis et al. (2016) used historical and modern samples of wild chipmunks separated by 100 generations, and showed that both the mean values of several cranium traits and the covariation between them had been altered by natural selection. Surprisingly, multivariate variation had increased in the direction of selection, suggesting that past selection can influence the standing covariation in a non-intuitive way, potentially facilitating further evolutionary responses in the same directions. These conflicting results regarding the interaction of multivariate variation and directional selection can be understood in light of recent theoretical work that allows for complex genetic architectures.

Heritable genetic covariation is determined by aspects of the genetic architecture like pleiotropy and linkage disequilibrium. Complex multivariate phenotypes like skeletal traits are influenced by a large number of loci, and covariation among these traits can be reasonably predicted from the pattern of shared pleiotropy (*i.e.* traits that have more loci affecting them simultaneously tend to be more correlated (Kenney-Hunt et al. 2008)). Porto et al. (2016) tested this hypothesis directly, comparing the level of pleiotropy between two species with different levels of phenotypic integration, and found that the more integrated species also showed higher levels of pleiotropy. In addition to pleiotropy, gene interactions (epistasis) can significantly influence covariation and alter patterns of pleiotropy (Pavlicev et al. 2008;

Wolf et al. 2005, 2006). Simulations using the information that epistatic interactions can provide variation in pleiotropy and covariance patterns, have shown how selection can promote changes in pleiotropy that can change covariation (Jones et al. 2014; Melo and Marroig 2015). Furthermore, Pavlicev et al. (2011) proposed a model for phenotypic evolution of multiple traits accounting for the influence of epistasis on the covariation between traits that shows how natural selection can increase the amount of variation along the direction of response to selection, exactly the kind of effect observed in Assis et al. (2016) and Roff and Fairbairn (2012).

In this article, we attempt to elucidate how directional selection interacts with and molds covariation using an experimental approach. Since selection on size is responsible for major morphological diversification (Baker et al. 2015), we used an evolutionary experimental approach in mice to investigate how evolutionary changes in a multivariate system of phenotypic traits alters the pattern and magnitude of association between these traits. Mice lines were selected for an increase and for a decrease in overall size, and we focused on the evolutionary consequences of the changes in standing covariation in the cranium. Under a traditional additive model of covariation, we expect selection to deplete variation in the direction of selection, while under a more complex genetic architecture, including epistatic variation in pleiotropy, we expect the variation to be reorganized and increased in the direction of selection. This experimental approach allows us to understand the maintenance and reorganization of variation in a complex system and its consequences to evolution.

3.2 Methods

3.2.1 Experimental selection lines

This study was performed using animals from a long term experiment involving one control line and two pairs of selected lines. In total, we used five groups: one control line t and four selected lines: upwards s', downwards s, upwards h' and downwards h. In 1985, a control

population t with an effective population size $Ne \approx 40$ was founded with breeders chosen at random from an CF1 outbred population of $Ne \approx 80$ at the Facultad de Ciencias Veterinarias (Universidad Nacional de Rosario, Argentina). Then, two line-pairs of two-way individual selection for body weight at 49 days of age (s and h: downwards selected lines; s' and h': upwards selected lines) were founded from control line t, with mice drawn from generation three for s and s' and from generation eight for h and h' (Fig. S3.1, Oyarzabal (2011), Renny et al. (2014)).

For control t line, effective population size was maintained by randomly choosing 20 individuals of each sex and avoiding full-sib mating. Selection on overall size was performed choosing the heaviest (for upwards lines) or lightest (for downwards lines) individuals for reproduction in each generation. Average effective population size of selected lines was maintained selecting six breeders of each sex for the downwards lines and four breeders of each sex for upwards lines. The difference in the number of breeders between upwards and downwards lines was due to the lower fertility of the lightest mice (Bernardi et al. 2009). Full-sib mating was also avoided in all the selected lines, except for the first generations of h and h' lines. For all matings females were exposed to males in the ratio of 1:1. All the animals were chosen regardless of their inbreeding coefficients and the selection differentials. After ≈ 50 generations the increase in inbreeding coefficients and the standard cumulative selection differentials were similar for selected lines (Table S3.1). See supporting information for additional information on number of weighted animals per generation (Fig. S3.2) and on average weight per generation (Fig. S3.3) for the full experiment.

3.2.2 Samples

We had access to ≈ 65 individuals from around the 50th generation of each line (for a total sample of 329), with well balanced sex ratios (see supporting information, Table S3.2 for details). Mice were euthanized by cervical dislocation according to the American Veterinary Medical Association 2007 Guidelines on Euthanasia. We prepared all specimens in a dermestarium and

removed their cranium and mandible. We collected 32 homologous anatomical landmarks in both sides of their cranium (Fig. S3.4, but see Cheverud (1995) and Garcia et al. (2014) for more details and the rationale for choosing these landmarks). In order to reduce measurement error, each anatomical marker was captured twice using a Microscribe MX 3D digitizer (Immersion Corporation – San Jose, California). We calculated a set of 35 euclidean distances (Fig. S3.4) between the landmarks using the average between both sides of the cranium for symmetrical distances, and the average between replicas of each individual. We opted for linear distances instead of following the current trend of using landmark data in a Geometric Procrustes Analysis (GPA) because GPA tends to disperse local variation and lead to misleading conclusions in regards to integration and modularity (see van der Linde and Houle (2009) and Márquez et al. (2012) for details and possible solutions inside a landmark approach) and so that our results would be comparable to other assessments of integration in the mammalian cranium (Porto et al. 2009, 2013).

3.2.3 Direction of phenotypic divergence

The multivariate phenotypic mean is a vector consisting of the mean of each cranial trait. In order to identify the direction in multivariate space for the phenotypic divergence between the two-way divergent selections, we calculated the vector of mean phenotypic divergence (δz). Each element of this vector was calculated as the difference between the pooled multivariate phenotypic mean of the lines selected for increase in weight and the pooled multivariate phenotypic mean of the lines selected for decrease in weight. We test if δz is indeed related to cranial size by comparing this vector with an isometric vector of equal loadings in all traits, which represents a direction of isometric size variation. High correlation between δz and the isometric vector indicates δz is related to cranial size. We also follow Mosimann (1970) and use the geometric mean of the cranial distances as a measure of overall isometric cranial size on an individual. This measure is highly correlated with the centroid size of cranial landmarks.

3.2.4 Covariance matrices

Phenotypic and additive genetic covariance matrices (P- and G-matrices) for cranial traits of each line were obtained using a Bayesian sparse factor mixed model (`BSFG`). This is a robust method for estimating high dimensional G- and P-matrices from limited samples proposed by Runcie and Mukherjee (2013) and implemented in Matlab (2013) by the authors. We removed differences due to age, generation and sex by using these groups as fixed effects in the mixed model. The models ran for 3000 iterations of burn in, followed by 100000 iterations with thinning interval of 100. Convergence was assessed by inspecting trace and auto-correlation plots. From this model, we obtained a posterior distribution of 1000 covariance matrices for each line that summarizes the uncertainty in the estimation of respective covariance matrices, and we used this distribution in all posterior analysis to generate posterior distributions for all the calculated statistics. This allows us to take uncertainty into account when comparing the lines. Default priors had little effect on the covariance matrices, and mean posterior matrices were similar to matrices from a traditional MANCOVA.

Here we use P-matrices as a proxy for the respective additive genetic matrices (G-matrix), which are the important parameter in multivariate evolution. This was done because when calculating the effective sample size of our pedigree using the approximations from Raffa and Thompson (2016) we arrived at very low effective samples: around one sibpair for each individual line and around ten sibpairs for the full pedigree. This means that the pedigree for the sample we measured is such that G-matrices for each line are estimated with far too much uncertainty to be useful (even when using the `BSFG` model). Fortunately, P-matrices are probably a better estimate of the underlying genetic covariance pattern (Marroig et al. 2012; Roff 1995) and are informative on the underlying pleiotropic structure of genetic effects (Kenney-Hunt et al. 2008; Porto et al. 2016). To test the validity of this approximation, we calculated a pooled within-group P-matrix and a pooled within-group G-matrix estimated by the `BSFG` model using all of the individuals and controlling for differences in means between the lines. We then compare these matrices to confirm that the P- and G-matrices are

similar. Matrix correlation between the pooled-within P- and pooled-within G-matrices was 0.95 for Random Skewers method (Cheverud and Marroig (2007), see supporting information, Table S3.3). Matrix correlation between the posterior mean P-matrices of each line and the pooled-within G-matrix were all above 0.86 (Table S3.3), supporting the idea that G and P are similar. This hypothesis of similarity between P and G has been tested several times for this set of traits, and has been shown to be quite accurate in rodents (Garcia et al. 2014), and in mammals overall (Cheverud 1988; Hubbe et al. 2016; Marroig and Cheverud 2010; Porto et al. 2009, 2016, 2015).

3.2.5 Matrix comparisons

Since we are interested in changes in the influence of the covariance patterns on evolution, we assess the overall level of similarity between the covariance matrices for all the lines using two comparison methods that have immediate evolutionary interpretations, the Bayesian versions of the Random Skewers method and Krzanowski method proposed in Aguirre et al. (2013). The Random Skewers method (Cheverud and Marroig 2007) uses the Lande equation to simulate the response to random selection gradients, and the responses to the same selection gradient are then compared using vector correlations (the cosine of the angle between them) for the two matrices being compared. Aguirre et al. (2013) uses these random responses to identify directions in which the set of matrices being compared differ in their amount of available variation. So, this method allows us to explore differences in the distribution of variation in multiple directions of the phenotype space. The Krzanowski method (Krzanowski 1979) measures how similar the spaces spanned by the first several eigenvectors of the matrices being compared. For two matrices, the Krzanowski correlation is the mean of the squared vector correlations between all pairs of the first eigenvectors, usually the first $\frac{n}{2} - 1$, where n is the dimensionality of the matrices. A Krzanowski correlation of 1 means the spanned spaces are exactly congruent, and a correlation of zero means the spaces are orthogonal. We can expand this method to several matrices by defining the **H** matrix (Krzanowski 1979):

$$H = \sum_{i=1}^p A_i A_i^t$$

where A_i is a column matrix containing the first $n/2 - 1$ eigenvectors of the i -th matrix being compared, p is the number of matrices being compared, and t denotes matrix transposition. The expectation of H is p times the covariance matrix of eigenvectors, and H/p tends to this covariance matrix for large p . The eigenvalues of \mathbf{H} are bounded by p , and any eigenvalue equal to p indicates the associated eigenvector can be reconstructed by a linear combination of the eigenvalues included in the A_i matrices, and so is shared by all the matrices. An eigenvalue of less than p indicates that at least one of the A_i matrices can not span that eigenvector, and so the space is not completely shared between all the matrices. In order to test if our matrices share the same subspace, we follow Aguirre et al. (2013) and construct a randomized set of matrices under the assumption that all matrices are sampled from the same population (see the supporting information in Aguirre et al. (2013)). Observed and randomized eigenvalues of \mathbf{H} are compared using posterior credibility intervals. If the randomized and observed eigenvalues of \mathbf{H} are the same, we conclude the matrices share the same subspace.

3.2.6 Evolutionary statistics

In order to assess the evolutionary consequences of the selection regimes on the covariance matrices, we calculated a series of evolutionarily informative statistics. In the following, \mathbf{G} is an arbitrary covariance matrix, \mathbf{G}^{-1} is the inverse of \mathbf{G} , $tr(\mathbf{G})$ is the trace of \mathbf{G} , $\lambda_i^{\mathbf{G}}$ is the i -th eigenvalue of \mathbf{G} , $\langle \cdot, \cdot \rangle$ represents the dot-product between two vectors, $\cos(\cdot, \cdot)$ is the cosine of the angle between two vectors (or their vector correlation), and $E[\cdot]_{\beta}$ represents the expected value over random β vectors with unit norm. (A) The magnitude of integration, calculated as the mean of the squared correlations between all traits. (B) The proportion of variation associated with the leading eigenvalue ($E1\% = \lambda_1^{\mathbf{G}}/tr(\mathbf{G})$). (C) The ability of the populations to respond in the direction of selection, calculated as the MEAN FLEXIBILITY (*sensu* Marroig et al.

(2009)), which is given by the mean vector correlation between random selection gradients and their respective expected response to selection given the Lande equation ($\bar{f} = E[\cos(\mathbf{G}\beta, \beta)]_\beta$). (D) The available variation for directional selection, calculated as the **MEAN EVOLVABILITY** (Hansen and Houle 2008), which is given by the mean projection of the responses to the random selection gradients on these same selection gradient ($\bar{e} = E[\langle \mathbf{G}\beta, \beta \rangle]_\beta$). The mean evolvability can also be calculated as the trace of the matrix being considered divided by the number of dimensions, so it is clearly a measure of total variation (Hansen and Houle 2008). Also, from the definition of the cosine between two vectors, flexibility in the direction of a given unit β can also be expressed as the ratio between evolvability in the direction of β and the norm of $G\beta$. We used a set of 1000 random selection gradients to calculate mean flexibility and mean evolvability. We then used these statistics to investigate how directional selection is affecting the evolutionary potential of each line.

We also evaluated how the direction of phenotypic divergence was related to the standing variation in the P-matrices. In this symmetrical directional selection case (*i.e.* where the two directions of selection are aligned but opposite) the direction of divergence δz describes the same direction as Δz . Furthermore, because our selection gradient is related to cranial size, the direction of phenotypic divergence is also a better predictor of the actual direction of selection than the selection gradient estimated indirectly from the response to selection and the G-matrix using the Lande equation (see supporting information for more details, and Marroig et al. (2012)). We used three directional metrics, (1) we calculated the ratio of the evolvability in the direction of the mean phenotypic divergence (with $\delta \hat{z}$ being the unit vector in the direction of divergence) and the mean evolvability along random phenotypic directions (**SCALED DIRECTIONAL EVOLVABILITY**) as a measure of how biased the variation is in the direction of phenotypic divergence ($\langle \mathbf{G}\delta \hat{z}, \delta \hat{z} \rangle / \bar{e}$); (2) similarly, we also measured the change in conditional evolvability, which measures the mean response to selection in the direction of a given β when other directions are under stabilizing selection (Hansen and Houle 2008). Mean conditional evolvability is calculated as $\bar{c} = E[(\langle \mathbf{G}^{-1}\beta, \beta \rangle)^{-1}]_\beta$, and we

define the ratio between conditional evolvability in the direction of phenotypic divergence and mean conditional evolvability along random phenotypic directions as the SCALED DIRECTIONAL CONDITIONAL EVOLVABILITY ($\langle \mathbf{G}^{-1}\delta\hat{z}, \delta\hat{z} \rangle^{-1} / \bar{c}$) (3) we compared the alignment between δz and the first eigenvector of the covariance matrix for each line, using vector correlations. This correlation is a measure of how aligned the main axis of variation in the population is with regards to the realized evolutionary change. Since the first eigenvector in mammalian cranial matrices is usually related to size variation (Porto et al. 2009) and selection was on overall size, we expect a high alignment between the phenotypic divergence and standing variation. We test if the first eigenvectors (E1s) are indeed related to cranial size by comparing the E1s of the mean posterior matrices of all lines with the isometric size vector. High correlation between the E1s and the isometric vector indicates the E1s are related to cranial size.

3.2.7 Data Availability

Code and data for performing all analysis are available from github (github.com/diogro/ratones, doi:10.5281/zenodo.815003), individual weight at 49 days and all cranial measurements, pedigree, posterior distribution, confidence intervals and mean of all P- and G-matrices are archived in Dryad (<http://dx.doi.org/10.5061/dryad.5gr8r>). Evolutionary statistics and matrix comparisons were calculated in R (R Development Core Team 2005) using the EvolQG package (Melo et al. 2015) (version 0.2-5).

3.3 Results

3.3.1 Phenotypic divergence

Both the upwards and the downwards selection resulted in changes in the weight of the corresponding s|s' and h|h' lines (Fig. S3.5 and S3.6). Both directional selection induced lower variation in weight, with the animals from the downwards selection showing smaller weights than those from the upwards selection, regardless of gender. In contrast, the control t line

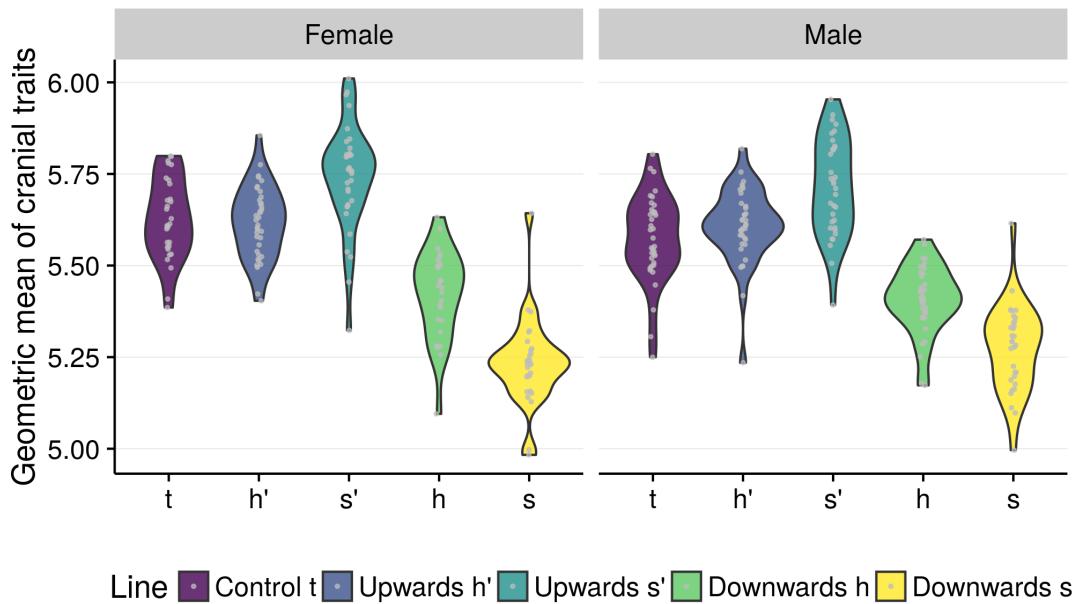


Figure 3.1: Distribution of the standardized isometric cranial size variable, calculated as the geometric mean of cranial traits by line and gender. Upwards lines are larger than downwards lines, while control line t is similar to the upwards lines.

exhibited a large weight variation, which spanned the full variation range presented by the selected animals. The cranial traits also showed divergence between all four selected lines, with downwards selection showing smaller cranial size than those from the upwards selection, and the control t line being somewhat superimposed with the upwards lines (Fig. 3.1 and S3.6 for the complete set of cranial traits). The vector of phenotypic divergence (δz) had a correlation of 0.82 with the isometric vector, indicating that divergence in cranial traits was mainly in the cranial size direction.

3.3.2 Matrix comparisons

In the Krzanowski subspace comparisons all eigenvalues were not significantly different between the observed and randomized matrix comparisons (Fig. 3.2A). As for the Bayesian Random Skewers projection, we identified several directions with different amounts of phenotypic variation in each line (first few directions in Fig. 3.2B and full results in Fig. S3.7). In

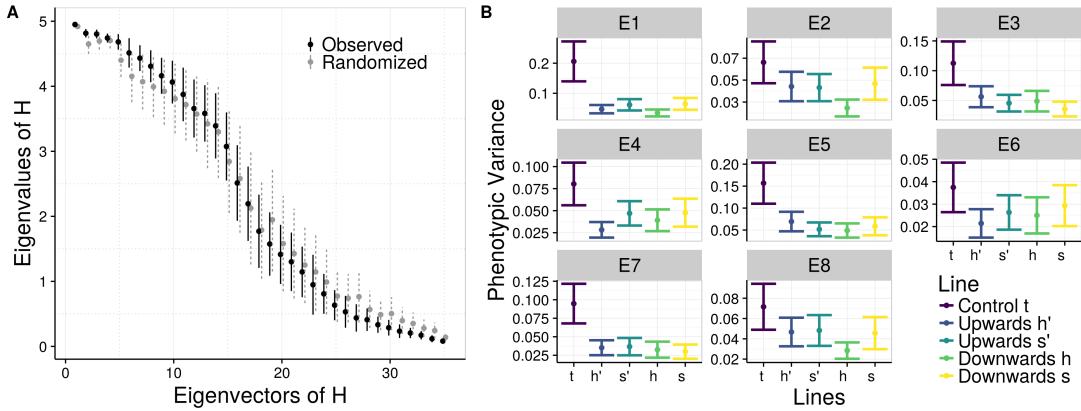


Figure 3.2: (A) Bayesian Krzanowski shared subspace. Eigenvalues for the H matrix are not significantly different in the randomized and observed matrices, indicating a shared subspace and a stable set of eigenvectors in all the lines. (B) Bayesian Random Skewers Projection. Here we only show the first eight eigenvectors of the decomposition, which are representative of the full set of eigenvectors (Fig. S3.7)). In most directions the control line has higher variation than the selected lines, but in several directions the control and selected lines show comparable levels of variation, indicating that the loss of variation in the selected lines was not uniform in all directions. A version of this figure using the G-matrices is available in the Supporting Information (Fig. S3.8)

most directions, the control line has significantly more variation than the selected lines. But this reduction is not uniform in all directions, and in several directions the selected lines have variation that is comparable to the control line. We provide in the supporting information the same set of results using G-matrices (Fig. S3.8). These results are considerably more noisy in several cases, but consistent with the results obtained using the P-matrices.

3.3.3 Evolutionary statistics

The distribution of mean values of evolutionary statistics showed a rise in integration and in the proportion of variation associated with the first eigenvector when compared to the control t line (Fig. 3.3A and B). All selected lines also showed a decline in evolvability and flexibility when compared to the control (Fig. 3.3C and D). First eigenvectors for each line are given in Table S3.4, along with correlations between the first eigenvector and an isometric size vector. All correlations between E1s and the isometric vector were higher than 0.71, indicating that all first eigenvectors are related with cranial size.

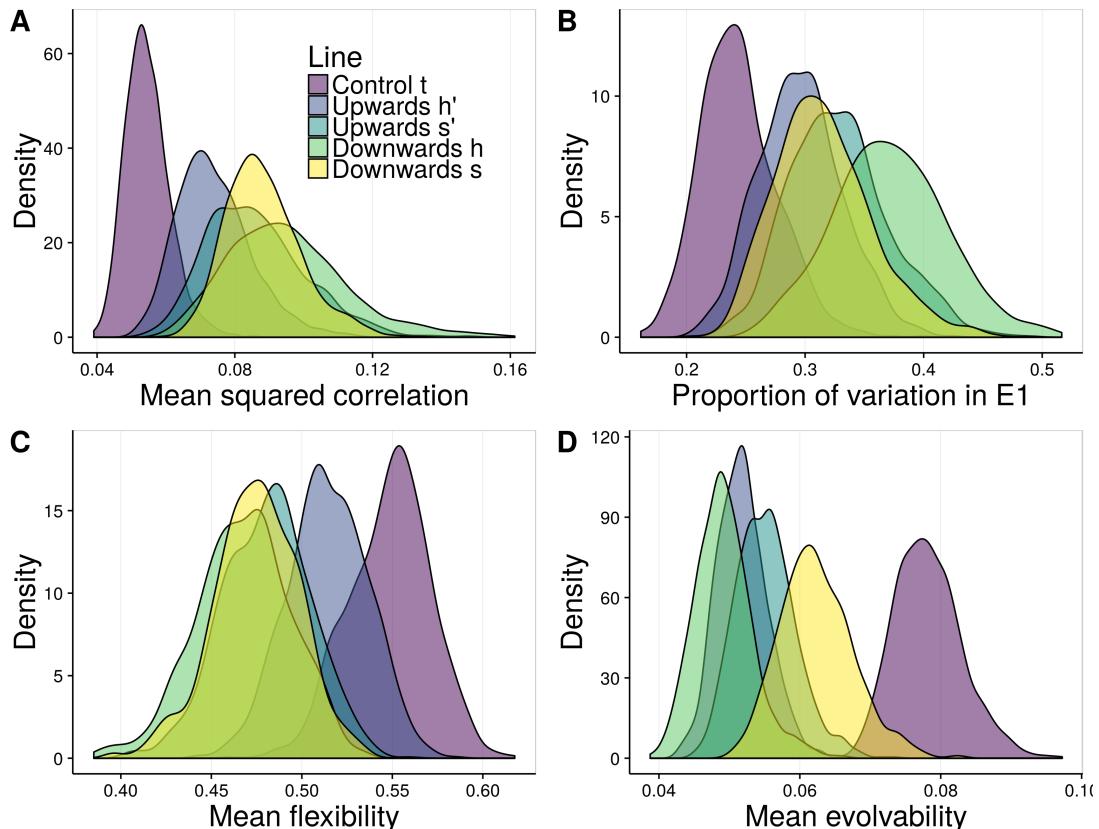


Figure 3.3: Comparison of covariation patterns between control and selected lines using evolutionary statistics (A) Magnitude of integration measured as mean squared correlation between all traits; (B) Proportion of variation associated with the first eigenvector, which is related to size variation; (C) Mean flexibility; (D) Mean evolvability. Curves represent posterior distributions obtained using the sample of P-matrices from the BSFG model. Confidence interval and mean for all curves can be found in Table S3.5. The same set of results using G-matrices are available in Fig. S3.9. G-matrix results are noisier, but consistent with the results obtained using the P-matrices, suggesting the changes we observed indeed occurred in the G-matrices.

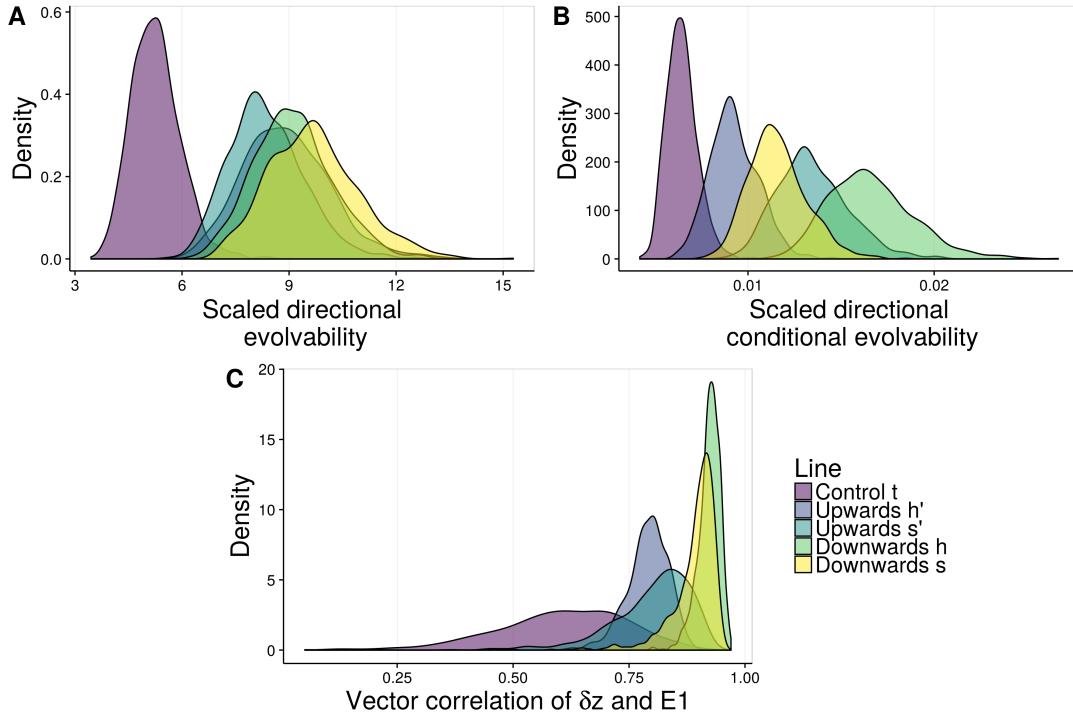


Figure 3.4: (A) Scaled direction evolvability, the ratio of evolvability in the direction of phenotypic divergence (δz) and the mean evolvability for each line; (B) Scaled direction conditional evolvability, the ratio of conditional evolvability in the direction of δz and the mean conditional evolvability for each line; (C) the vector correlation between δz and first eigenvector for each line. Curves represent posterior distributions obtained using the sample of P-matrices from the BSFG model. Confidence interval and mean for all curves can be found in Table S3.6. The same set of results using G-matrices are available in Fig. S3.10. G-matrix results are noisier, but consistent with the results obtained using the P-matrices, suggesting the changes we observed indeed occurred in the G-matrices.

The scaled directional evolvability, the ratio of evolvability in the direction of δz and the mean evolvability, shows a clear increase with selection, with selected lines showing about double the ratio observed in the control line (Fig. 3.4A). The vector correlation between δz and the E1 increased in all selected lines, while this correlation in the control line shows a lower mean correlation (around 0.6) and wider posterior distribution. Downward selected lines have correlations of first eigenvector and δz above 0.9 and upwards selected lines above 0.75 (Fig. 3.4B).

3.4 Discussion

Our experimental approach allows us to investigate how directional selection alters multivariate covariation, and what are the consequences of this interaction to evolution and diversification. Selected lines diverged in both weight and cranial traits, and the changes in the cranium were aligned with the main axis of variation, which is a direction associated with cranial size. While to some extent this is an unsurprising result given that selection was on overall size, our results illustrate how rapidly covariation can change under directional selection. In particular, the magnitude of association between traits is more malleable than the pattern of association.

Patterns of covariation were fairly similar in all lines, a result consistent with the observed pattern for natural populations of mammals in general, even when comparing covariation between different orders (Porto et al. 2009). Krzanowski subspace comparison showed all the lines share the subspace spanned by the first half of the eigenvectors. Regarding the distribution of variation, the Bayesian Random Skewers showed that in some directions the control line has more variation than the selected lines, while in others control and selected lines have comparable levels of variation. Taken together, these matrix comparison results indicate a stable set of eigenvectors, spanning a similar space in all the lines, and a non-isotropic reduction in variation in the selected lines, that is, some directions lost more variation than others (Figs. 3.2 and S3.7)).

As for integration, all selected lines increased their magnitude of association between traits. The maximum observed difference in mean squared correlation is almost 0.05 (when comparing the control t line and the downwards h line) a difference comparable to those observed between mammalian orders (Marroig et al. 2009). This increase in integration is mirrored by the observed increase in the proportion of variation associated with the first eigenvector, which is related to size. This is expected, because size variation can be interpreted as coordinated variation in all traits, and so an increase in size variation leads to higher correlations between all traits (Porto et al. 2013). Therefore, the rise in integration in the

selected lines is due to an increase in the proportion of variation that is related to cranial size (which changed due to selection on overall size).

This increase in integration is also reflected in the flexibility, which is lower in all the selected lines. Flexibility measures the ability of a population to respond in the direction of selection, and because a larger proportion of cranial variation in the selected lines is concentrated in size variation, less variation is available to respond in other directions. Also, the mean evolvability, or total available variation for responding to selection, is smaller in the selected lines.

At first sight, this is compatible with the idea that directional selection depletes variation. However, focusing only on the reduction of total variation is misleading. Taking the multivariate aspect of this system in consideration, the distribution of the available variation is also changing. Although total variation in all directions is smaller in the selected lines (Fig. 3.3, panel D), the increase in the proportion of variation associated with the first eigenvector (Fig. 3.3, panel B) (which is highly related to cranial size, Table S3.4) means the proportion of variation in the direction of evolutionary divergence is in fact increasing in the selected lines (Fig. 3.3, panels A and B), and the first eigenvector is also more aligned with the direction of divergence in the selected lines (Fig. 3.4C). This reorganization of variation is supported by the change in the scaled directional evolvability and scaled conditional evolvability, which show that the scaled variation in the direction of divergence is almost doubled in selected lines when compared to the control (Fig. 3.4A and B).

This relative increase in variation is incompatible with the traditional view of depletion of variation in the direction of selection due to the fixation of additive alleles. Under a purely additive model of cranial variation, as rare additive alleles with effects on size move to fixation, they could increase genetic variation for size in the process (Bürger and Lynch 1995). In large populations this increase in variation under directional selection can be maintained by new mutations that arise and sweep to fixation. In principle we can't rule out this possibility, but we are observing this effect after about 50 generations of strong directional selection (Table S3.1),

after which we would expect the initial increase of variation to have disappeared as those initially rare alleles become fixed, and, because of our low effective population sizes, the influx of new mutations should be negligible. Also, increase in size variation due to additive alleles would be more likely to occur in traits that are controlled by a small number of loci (Bürger and Lynch 1995; Jain and Stephan 2015), which is not likely to be the case for the cranium (Leamy et al. 1999; Porto et al. 2016; Wolf et al. 2005). Furthermore, the genetic basis of morphological covariation is unlikely to be purely additive (Phillips et al. 2001; Whitlock et al. 2002). The changes are also not likely to be due to drift alone, as all the selected lines are consistent in their covariation patterns, and under drift we would expect a more random distribution of differences between the lines. However, epistatic interactions can provide a source of standing variation that allows the increase of variation in the direction of selection (Cheverud and Routman 1995; Pavlicev and Hansen 2011; Wagner et al. 2007), and can bias further mutations to be aligned with this direction (Jones et al. 2007, 2014).

Because genetic architectures can interact with selection in different ways, we expect different outcomes depending on the complexity of the genetic architecture underlying the set of traits under investigation: additive variation is expected to be consumed in the direction of selection, while epistatic variation can lead to an increase in variation in the direction of selection. Careau et al. (2015) showed that directional selection on behavior traits follows the expectation of the additive model, with a loss of variation in the direction of selection and a plateau in the response to selection after a few generations. In our experiment, as expected by the additive model, we also see a loss of total variation in the selected lines, but this reduction is anisotropic. Some directions lost more variation than others, and the direction of selection ended up with proportionally more variation in the selected lines, suggesting the influence of epistasis and a more complex genetic architecture underlying the covariation of cranial traits. Assis et al. (2016) reported the same kind of realignment of variation and selection as we did, but saw no loss of variation in samples from modern populations after selection, when compared to historical samples. This could be related to the large effective sample sizes in the

wild chipmunk populations considered, and suggests that large populations can reorganize covariation patterns in response to selection with no loss in total variation. Neither our results nor those from Assis et al. (2016) allow us to evaluate limits in the response to selection in the cranium, leading to plateaus in response, but we speculate that in large populations the availability of standing epistatic variation and the biasing of new mutations in the direction of selection could delay the onset of the kind of plateau in multivariate evolutionary response seen in Careau et al. (2015).

The observed increase in integration over a microevolutionary time scale also has consequences for our understanding of macroevolutionary patterns. In mammals, changes in the magnitude of integration are much more common than changes in the pattern of trait association (Porto et al. 2009). Our results suggest that these difference can be explained by the pervasiveness of selection on size along the mammalian clade (Baker et al. 2015; Marroig and Cheverud 2005). Lineages that underwent selection on size might have higher integration, and those whose selective response were not size-related might have lower integration. Also, divergence that is aligned with covariation can not be interpreted as only a product of constraints, since selection can directly reshape variation (Punzalan and Rowe 2016).

Here we attempt to elucidate the effect of directional selection on covariation, and how this impacts evolution. We use experimental selection on size to answer this question, and find that selection can actively restructure covariation, and, in addition of depleting multivariate covariation, can reorganize standing covariation in the direction of evolutionary response, increasing a population's relative ability to respond to selection in that direction. This is in accordance with recent models of phenotypic covariation that include a more realistic genetic architecture, and an experimental evidence of directional selection shaping variation in non-intuitive ways. Along with recent empirical, theoretical, and simulation work, our results reinforce a shift in our understanding of how populations are shaped by natural selection. An obvious next step is to combine this sort of experimental selection with genetic mapping to understand, at the genomic level, how this reorganization of variation is taking place.

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Author Contributions

GM and MO designed the research. MO and SB conducted the selection experiments. AP cleaned and measured all animals. AP, DM and GM designed the analysis. AP and DM performed the analysis and wrote the initial draft. AP, DM, MO and GM reviewed and edited the final manuscript.

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Supporting Information

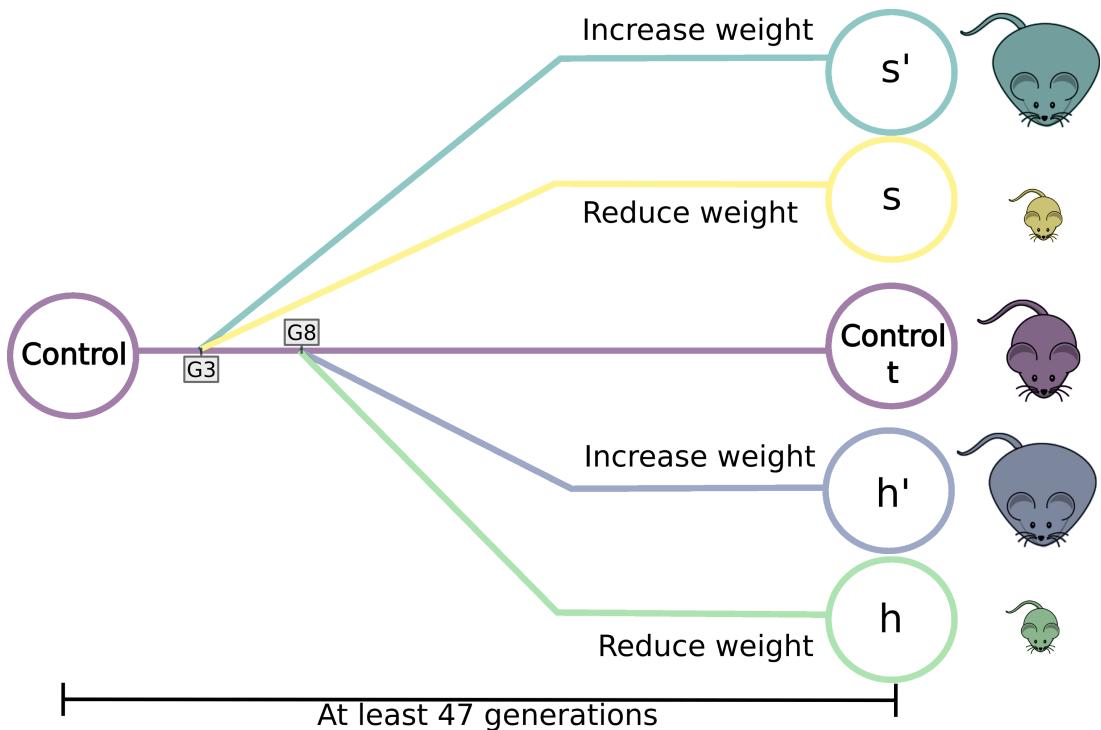


Figure S3.1: Scheme of relationship between mice lines used in the study. Selection regime for body weight at 49 days was conducted in s and h lines for reduction, in s' and h' lines for increase, and a control line was maintained with random mating along generations, but avoiding full-sib matings. In downwards s and upwards s' lines inbreeding was performed by limiting population size and in downwards h and upwards h' full-sib mating was performed only during first generations.

Table S3.1: Number of animals per generation, inbreeding coefficients and selection differentials by line. N average number of weighted individuals per generation. Ne effective population size. %d percentage of animals that left descendants. F_{50} increase in inbreeding coefficients for each line since the foundation, calculated as $F_G = \frac{1}{2N} + (1 - \frac{1}{2N})F_{G-1}$. Standard cumulative selection differential (SD) at 50th generation per sex for each line, calculated as the sum of selection differential over 50 generations divided by the standard deviation.

Selection	Line	N	Ne	%d	F_{50}	$SD_{females}$	SD_{males}
Control	t	162	40	24	0.48	2.5	6.9
Downwards	h	59	12	16	0.91	-15.1	-23.1
Downwards	s	59	8	20	0.91	-24.3	-25.6
Upwards	h'	69	12	12	0.93	43.3	47.0
Upwards	s'	74	8	14	0.93	45.8	46.8

Table S3.2: Sample sizes by line and sex.

Selection	Line	Generations	Females	Males	Total
Control	t	55 th	30	37	67
Downwards	h	47 th , 48 th	27	35	62
Downwards	s	52 th , 53 th	28	31	59
Upwards	h'	47 th	37	36	73
Upwards	s'	52 th	34	34	68

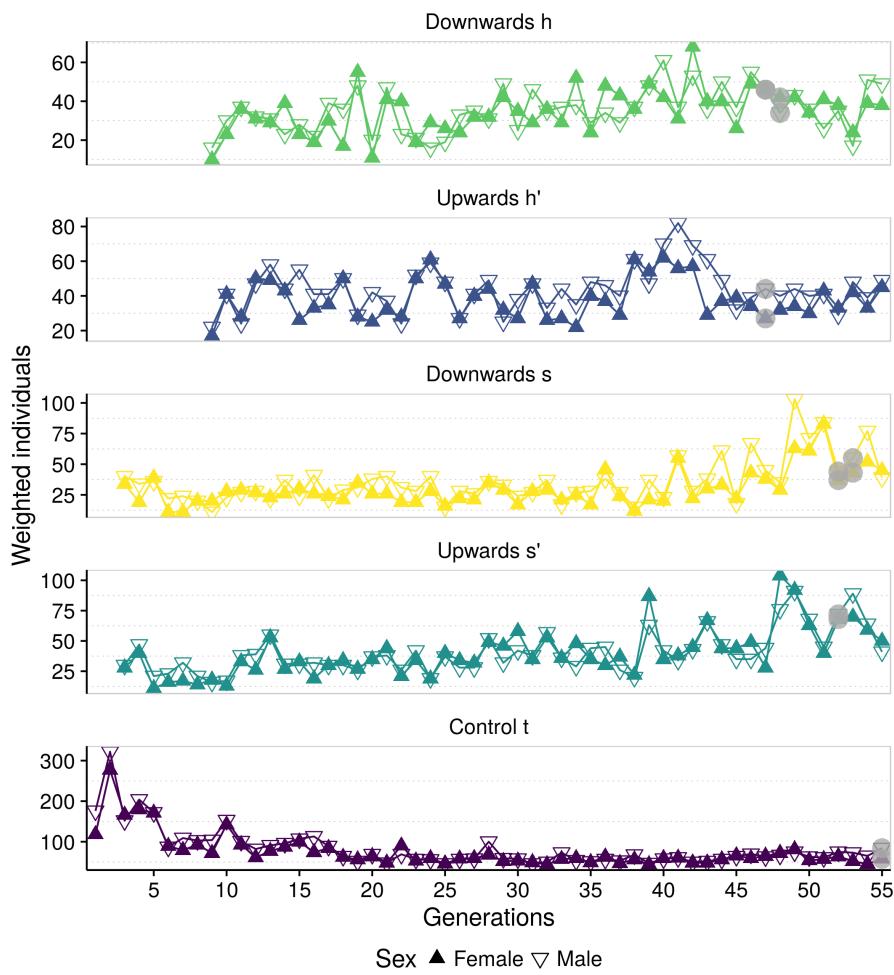


Figure S3.2: Number of weighted animals per generation by line and sex. Grey circles highlight the generations used in this study.

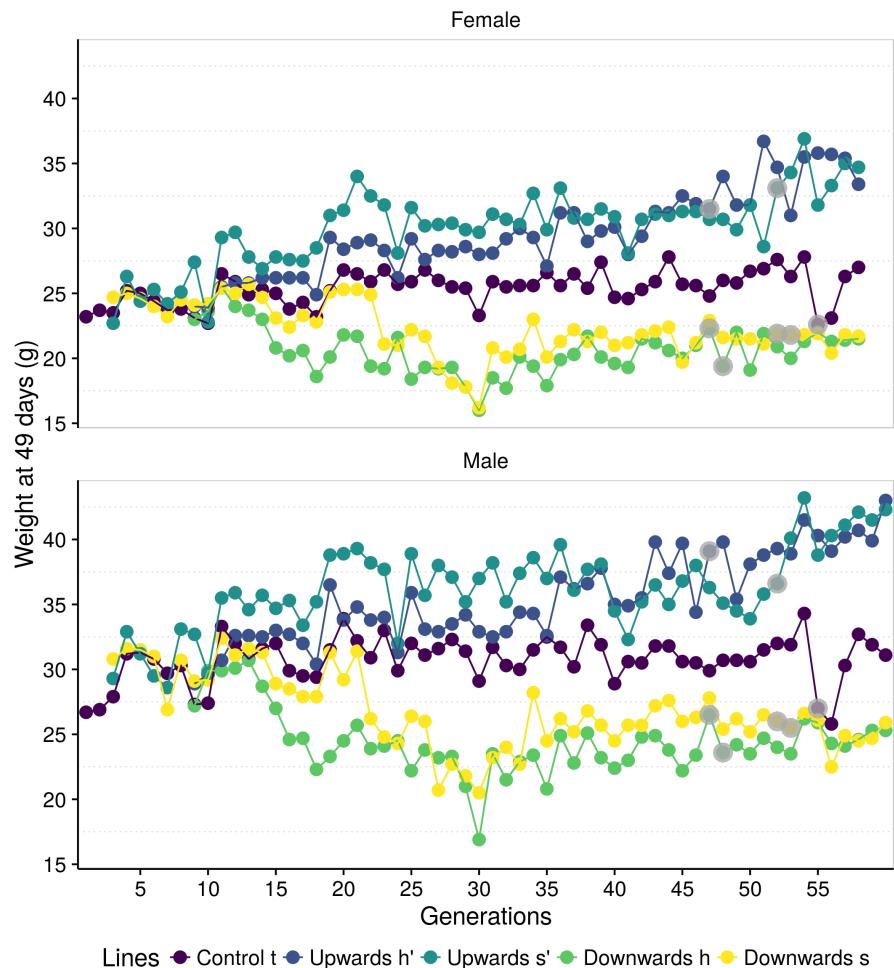


Figure S3.3: Average weight at 49 days per generation by line and sex, for the full experiment. Grey circles highlight the generations used in this study. We can see that after around the first 15 generations, besides some fluctuations, all selected lines consistently responded to directional selection, being the upward selected line heavier than control t line and downward selected lines lighter than the control t line. After the 56th generation, the mean weight of control t line was similar to that of the previous generations.

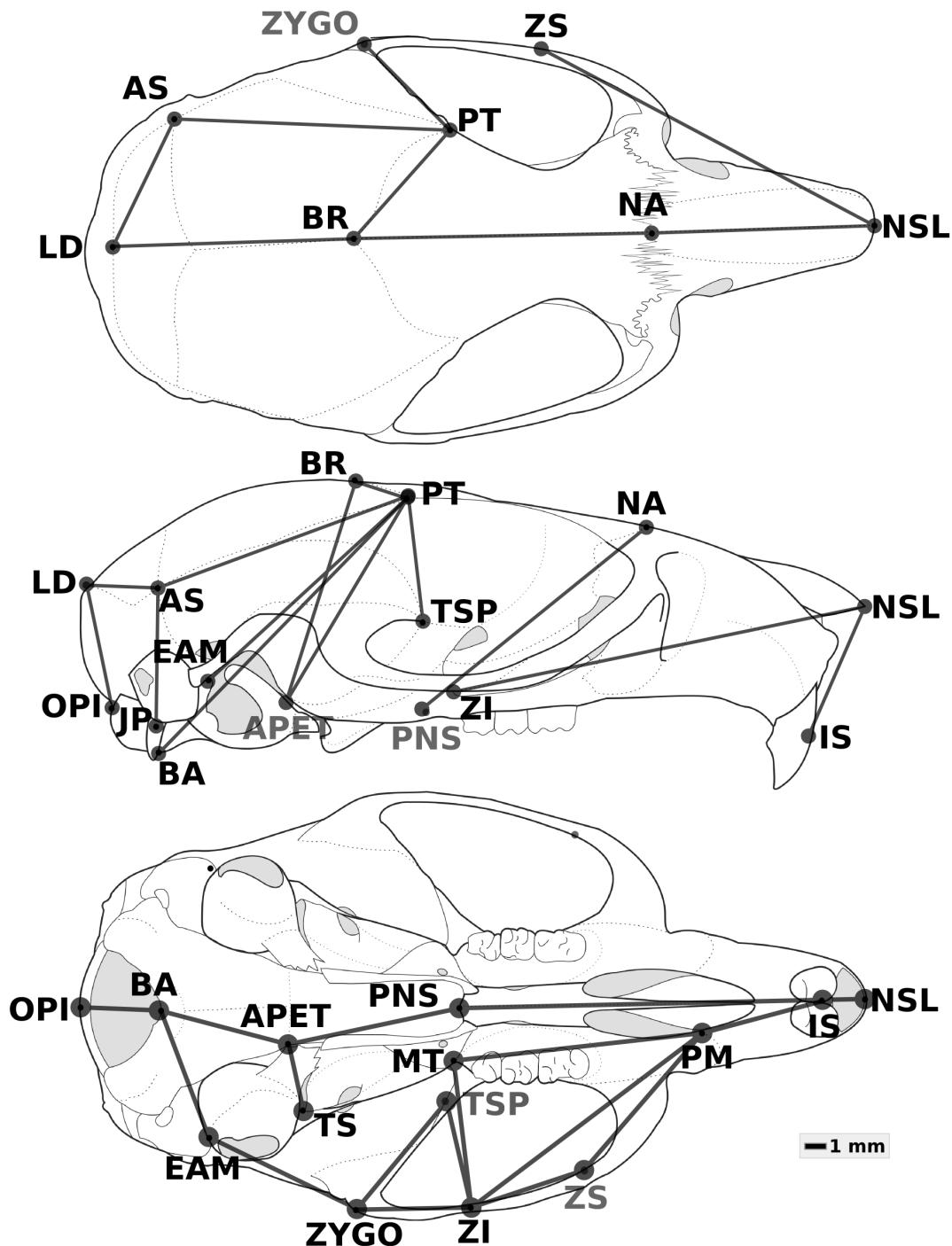


Figure S3.4: Cranial anatomical landmarks used in this study and set of euclidean distances calculated between them, following Cheverud (1995). Illustrations by Julia Laterza Barbosa.

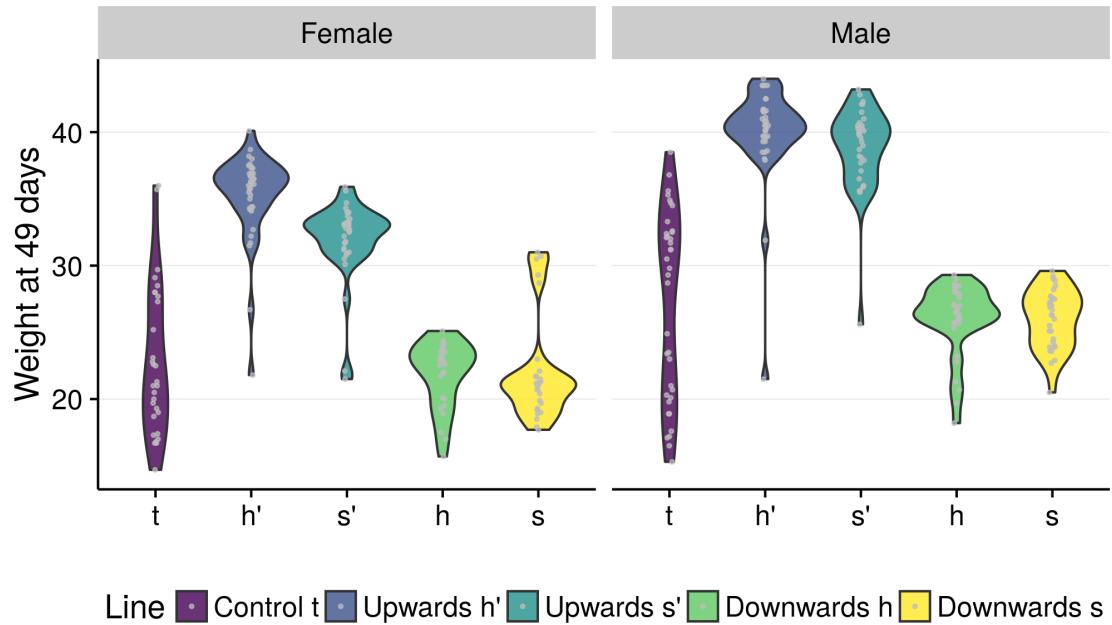


Figure S3.5: Distribution of weight by line and sex. Selected lines have much lower variation, and upwards lines are larger than downwards lines, while control line has very large variance.

Table S3.3: Comparison between the posterior mean G-matrix and all posterior median P-matrices and within groups P-matrix.

Selection	Line	Random Skewers	Krzanowski
Control	t	0.84	0.80
Downwards	h	0.85	0.77
Downwards	s	0.83	0.76
Upwards	h'	0.84	0.80
Upwards	s'	0.88	0.78
Within Groups P-matrix	all	0.95	0.86

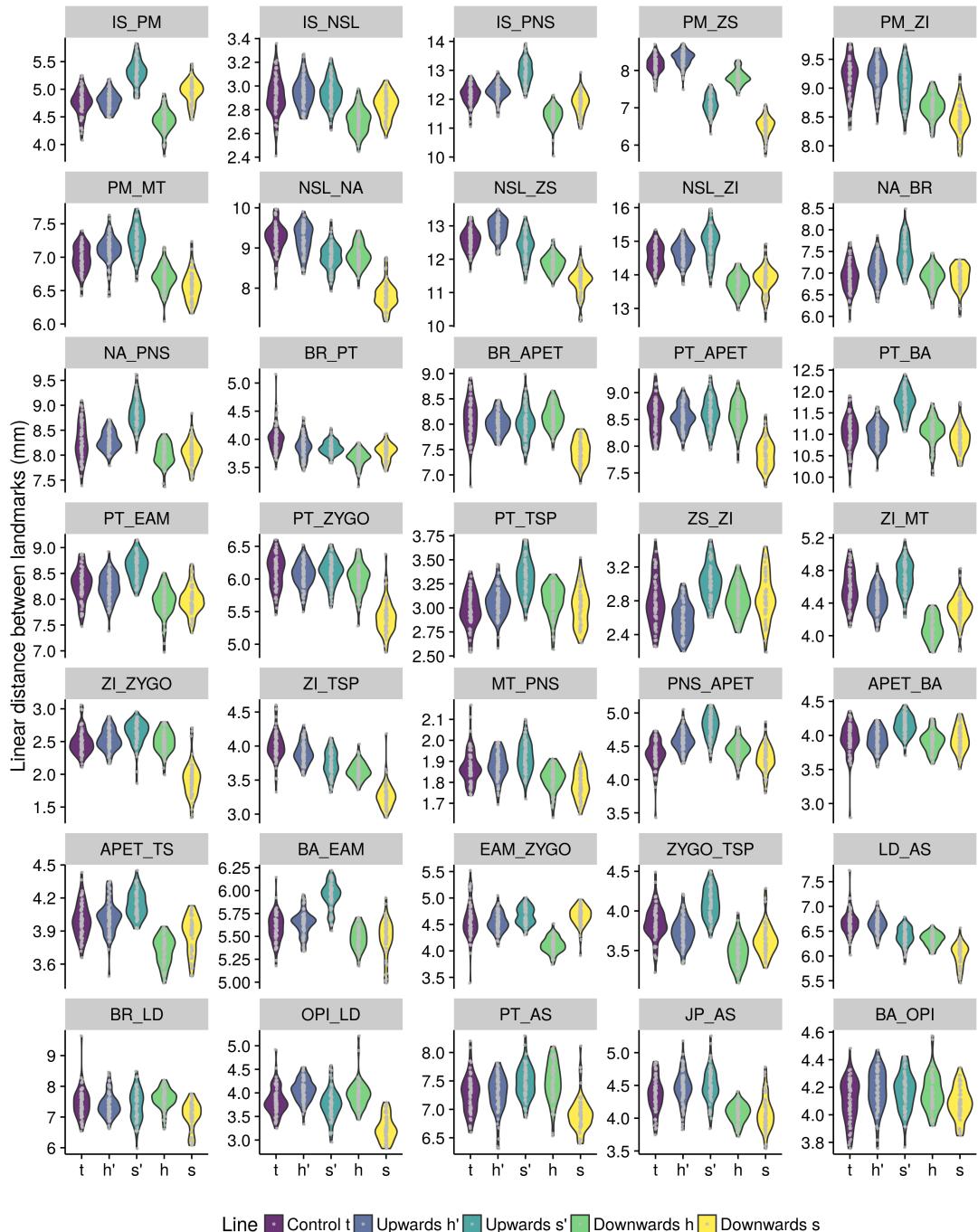


Figure S3.6: Distribution of all inter-landmark distances used in this study. In most cases, upwards lines are larger than downwards lines, but there are exceptions in the h line measurements, probably due to drift.

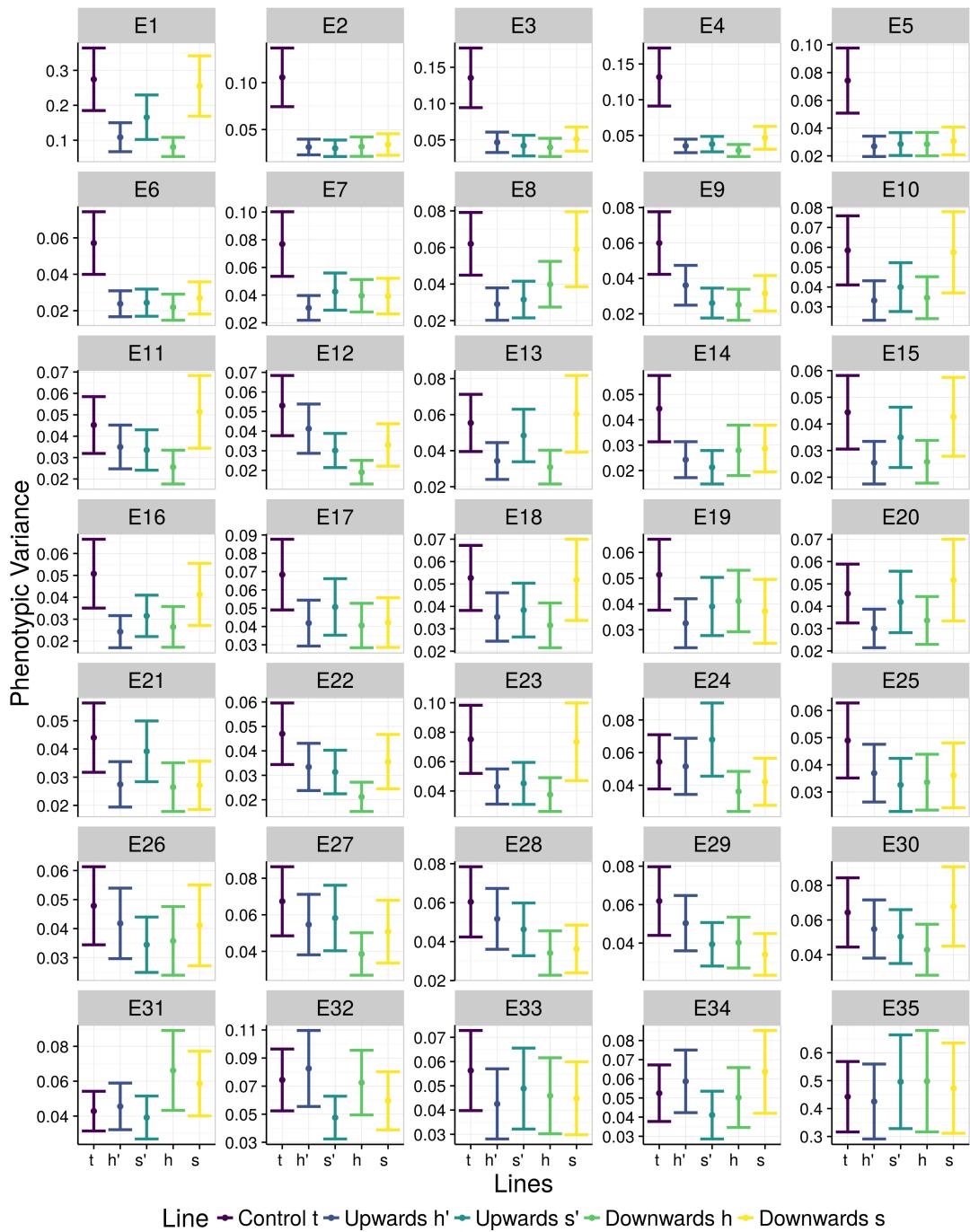


Figure S3.7: Bayesian Random Skewers results for all 35 dimensions.

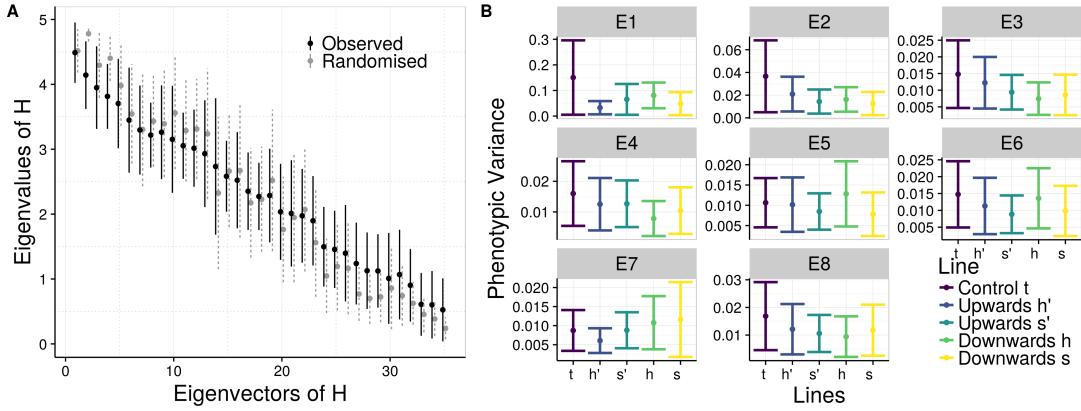


Figure S3.8: (A) Krzanowski shared subspace; (B) Bayesian Random Skewers. Results obtained using the sample of G-matrices from the BSFG model.

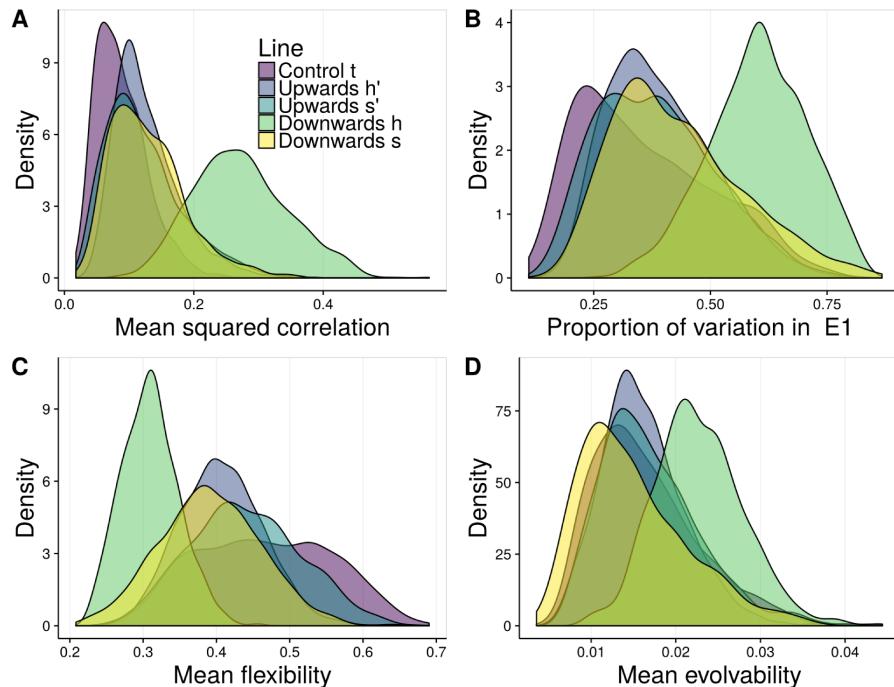


Figure S3.9: Comparison of covariation patterns between control and selected lines using evolutionary statistics (A) Overall integration measured as mean square correlation between all traits; (B) Proportion of variation associated with the first eigenvector, which is related to size variation; (C) Mean flexibility; (D) Mean evolvability. Curves represent posterior distributions obtained using the sample of G-matrices from the BSFG model.

Table S3.4: First eigenvectors (E1) for the posterior median P-matrix for each line, and correlation of the E1s with an isometric size vector (a vector with equal loadings in all traits). Most loadings are positive or small, and correlation with isometric size vector is high (above 0.78) for all strains, indicating that all E1 are size related.

	Control t	Upwards h'	Upwards s'	Downwards h	Downwards s
IS_PM	0.151	-0.173	-0.170	-0.104	0.089
IS_NSL	0.007	-0.020	-0.029	-0.033	0.020
IS_PNS	0.200	-0.343	-0.318	-0.326	0.294
PM_ZS	0.110	-0.093	-0.145	-0.201	0.209
PM_ZI	0.039	-0.134	-0.254	-0.294	0.247
PM_MT	0.058	-0.131	-0.154	-0.178	0.194
NSL_NA	0.087	-0.191	-0.112	-0.226	0.155
NSL_ZS	0.091	-0.172	-0.346	-0.336	0.318
NSL_ZI	0.160	-0.246	-0.419	-0.396	0.350
NA_BR	0.055	-0.065	-0.142	-0.258	0.156
NA_PNS	0.363	-0.184	-0.016	-0.138	0.158
BR_PT	-0.032	-0.069	-0.009	-0.056	0.020
BR_APET	0.378	-0.200	-0.028	-0.094	0.251
PT_APET	0.366	-0.312	-0.165	-0.184	0.220
PT_BA	0.425	-0.381	-0.213	-0.251	0.291
PT_EAM	0.272	-0.325	-0.144	-0.248	0.194
PT_ZYGO	0.239	-0.237	-0.104	-0.161	0.182
PT_TSP	0.113	-0.121	-0.011	-0.063	0.080
ZS_ZI	0.224	-0.091	-0.014	-0.018	0.026
ZI_MT	0.018	-0.067	-0.143	-0.133	0.065
ZI_ZYGO	-0.022	-0.034	-0.021	-0.002	0.071
ZI_TSP	0.075	-0.074	-0.030	-0.077	0.104
MT_PNS	0.024	-0.037	-0.003	-0.024	0.034
PNS_APET	0.081	-0.105	-0.150	-0.099	0.130
APET_BA	0.073	-0.061	-0.070	-0.096	0.082
APET_TS	0.039	-0.050	-0.113	-0.070	0.045
BA_EAM	0.058	-0.080	-0.176	-0.084	0.094
EAM_ZYGO	0.087	-0.091	0.015	-0.087	0.039
ZYGO_TSP	0.044	-0.112	0.003	-0.092	0.107
LD_AS	0.021	-0.050	-0.226	-0.055	0.079
BR_LD	0.067	-0.195	-0.382	-0.025	0.192
OPI_LD	0.123	-0.002	0.083	-0.081	0.171
PT_AS	0.195	-0.263	-0.080	-0.145	0.150
JP_AS	0.018	-0.063	-0.154	-0.120	0.167
BA_OPI	-0.007	0.030	0.008	0.000	0.026
Correlation with isometric vector	0.71	0.80	0.72	0.85	0.85

Table S3.5: 95% confidence interval and mean of the curves of posterior distributions obtained using the sample of P-matrices from the BSFG model from Fig. 3.3.

	variable	selection	line	ic.min	mean	ic.max
A. Mean Squared Correlation	control	t	0.04	0.05	0.07	
	upwards	h'	0.06	0.07	0.10	
	upwards	s'	0.06	0.09	0.12	
	downwards	h	0.07	0.10	0.14	
	downwards	s	0.07	0.09	0.11	
B. Proportion of Variation in E1	control	t	0.19	0.24	0.31	
	upwards	h'	0.23	0.30	0.37	
	upwards	s'	0.26	0.33	0.42	
	downwards	h	0.28	0.37	0.47	
	downwards	s	0.25	0.32	0.41	
C. Mean Evolvability	control	t	0.07	0.08	0.09	
	upwards	h'	0.05	0.05	0.06	
	upwards	s'	0.05	0.06	0.07	
	downwards	h	0.04	0.05	0.06	
	downwards	s	0.05	0.06	0.07	
D. Mean Flexibility	control	t	0.50	0.55	0.59	
	upwards	h'	0.47	0.51	0.56	
	upwards	s'	0.43	0.48	0.53	
	downwards	h	0.41	0.47	0.52	
	downwards	s	0.42	0.47	0.52	

Table S3.6: 95% confidence interval and mean of the curves of posterior distributions obtained using the sample of P-matrices from the BSFG model from Fig. 3.4.

	variable	selection	line	ic.min	mean	ic.max
A. Scaled directional evolvability	control	t	4.10	5.26	6.73	
	downwards	h	6.74	8.92	11.59	
	downwards	s	6.58	8.36	10.64	
	upwards	h'	7.04	9.11	11.48	
	upwards	s'	7.37	9.68	12.43	
B. Scaled directional conditional evolvability	control	t	0.01	0.01	0.01	
	downwards	h	0.01	0.01	0.01	
	downwards	s	0.01	0.01	0.02	
	upwards	h'	0.01	0.02	0.02	
	upwards	s'	0.01	0.01	0.02	
C. Vector correlation with E1	control	t	0.30	0.61	0.84	
	downwards	h	0.69	0.79	0.86	
	downwards	s	0.56	0.80	0.91	
	upwards	h'	0.87	0.92	0.96	
	upwards	s'	0.76	0.89	0.94	

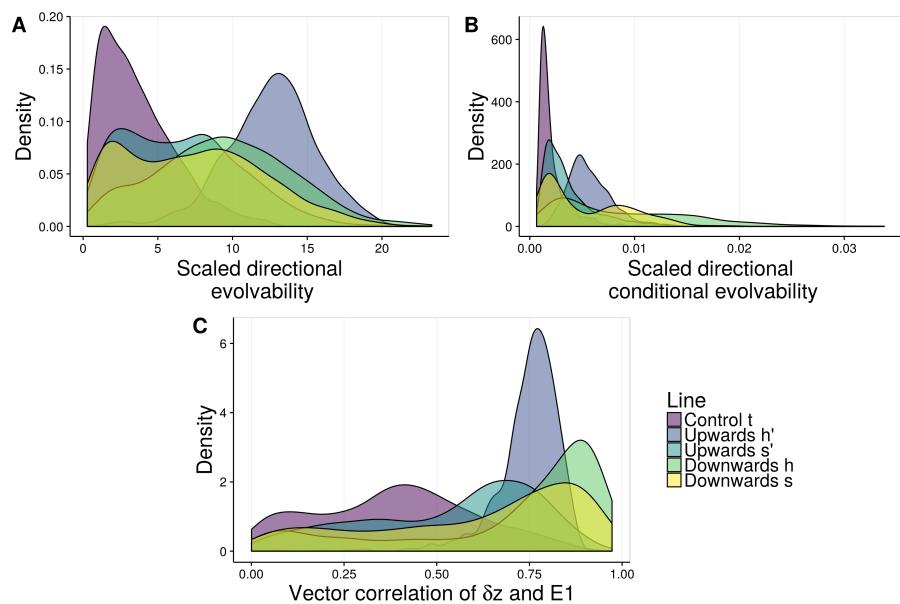


Figure S3.10: (A) Scaled direction evolvability, the ratio of evolvability in the direction of δz and the mean evolvability for each line; (B) Scaled direction conditional evolvability, the ratio of conditional evolvability in the direction of δz and the mean conditional evolvability for each line; (C) the vector correlation between δz and first eigenvector for each line. Curves represent posterior distributions obtained using the sample of G-matrices from the BSFG model.

Chapter 4

Genomic Perspective On Multivariate Variation, Pleiotropy, And Evolution

Diogo Melo, Gabriel Marroig, Jason Wolf

Abstract

Multivariate quantitative genetics provides a powerful framework for studying patterns and processes of phenotypic evolution. Quantitative genetics parameters, like trait heritability or the G-matrix for sets of traits, can be used to predict evolutionary response or to understand the evolutionary history of a population. These population-level approaches have proven to be largely successful, but the underlying genetics of multivariate variation and evolutionary change typically remain a black box. Establishing a deeper empirical understanding of how individual genetic effects lead to genetic (co)variation is then crucial to our understanding of the evolutionary process. To delve into this black box, we exploit an experimental population of mice composed from lineages derived by artificial selection for body size. We develop an approach to estimate the multivariate effect of loci and characterize these vectors of effects in terms of their magnitude and alignment with the direction of evolutionary divergence. Using these estimates, we reconstruct the traits in the ancestral populations and quantify how much of the divergence is due to the different kinds of genetic and environmental effects. Finally, we also use these vectors to decompose patterns of genetic covariation and examine the relationship between these components and the corresponding distribution of pleiotropic effects. We find that additive effects are much larger than dominance effects and are more closely aligned with the direction of selection and divergence. Pleiotropic effects are highly variable but are, on average, modular. These results are consistent with pleiotropy being partly shaped by selection, while reflecting underlying developmental constraints on patterns.

Keywords: G-matrix; QTL mapping; genome prediction; genetic architecture

4.1 Introduction

Individuals are composed of a complex array of traits that are interconnected through shared genetic, physiological, and developmental processes. Consequently, evolution is inherently a ‘multivariate’ process, wherein suites of traits interact to determine an individual’s fitness. Differences in fitness then generate selection that cascades to the genomic level through the genotype-phenotype relationship, leading to heritable changes across generations (Klingenberg 2008; Lande and Arnold 1983; Melo et al. 2016). Therefore, understanding evolutionary change in response to selection requires deciphering the relationship between genotypic variation and multivariate traits. The quantitative genetics framework was developed to achieve this goal, historically relying on statistical measurement of genetic covariation between traits as a summary of their genetic ‘connectedness’. The covariances estimated using this framework can be used to describe how sets of traits evolve together, including either for predicting the multivariate response to selection (Lande 1979), or to retrospectively analyze divergence by selective and neutral processes (Felsenstein 1988). However, this statistical framework treats the link between genotype and phenotype underlying patterns of covariation as a ‘black box’, and as a result, we still have a relatively limited understanding of the underlying genetic architecture of patterns of genetic covariation. Furthermore, patterns of genetic covariation are themselves shaped by selection, which can lead to a complex feedback between genetic architecture and evolution which we don’t fully understand (Jones et al. 2004, 2014; Turelli and Barton 1994). Therefore, an explicit link between the properties of the individual loci that underlie the multivariate genotype-phenotype relationship, and the associated consequences for patterns of genetic covariation are central to the study of evolution.

To understand the patterns of genetic covariation between traits, studies have historically taken a population level approach (top-down) that relies on pedigree relations to dissect components of phenotypic covariation. These studies are generally focused on how variation constrains evolution or how variation itself evolves in relation to multivariate selection

(Arnold et al. 2008; Futuyma 2010). Whereas the effect of covariation in constraining short term evolution is well established (Grant and Grant 1995; Lande 1979; Schluter 1996), we have only recently begun to uncover the effects of covariation on macroevolution. Comparisons of directions of divergence in several study systems have shown a remarkable pattern of alignment between within population covariation and macro- evolutionary divergence, with divergence being greater in directions of high genetic variation, both in patterns of phenotypic (Marroig and Cheverud 2005; Punzalan and Rowe 2016; Simon et al. 2016) and genetic covariation (Houle et al. 2017; McGlothlin et al. 2018). Whether this alignment between divergence and covariation is a result of macroevolutionary constraints limiting possible directions of evolutionary change, or due to selection actively changing constraints using the available genetic variation in patterns of covariation (Arnold et al. 2001; Pavlicev and Cheverud 2015; Pavlicev and Hansen 2011; Walter et al. 2018), or a mixture of both, is still an open question. The extent to which genetic covariation can evolve has been investigated in short term artificial (and natural) selection experiments, that have shown that the G-matrix can quickly evolve in response to selection (Assis et al. 2016; Careau et al. 2015; Penna et al. 2017), but there are also examples of failure to increase multivariate variation via selection (Sztepanacz and Blows 2017). Given this rich scenario of experimental and natural observations, a qualitative exploration of the sources and causes of genetic covariation, and on how these constraints evolve, is fundamental to furthering our understanding of diversification. Patterns of genetic covariation between traits can be due to either pleiotropy (where a locus affects more than one trait), or linkage disequilibrium (LD) (where alleles at different loci affecting different traits tend to be co-inherited). Studies generally assume the primacy of pleiotropy as a cause of long term genetic covariation, because even relatively closely linked genes are expected to reach linkage equilibrium over evolutionary timescales, and hence even when LD is present, its influence is likely to erode (unless actively maintained by selection).

Although the potential causes of covariation are known, the population level top-down approach only brings us indirect evidence of their contribution to the structure and evolution

of genetic covariation, and therefore we must then rely on theoretical and computational models to infer how pleiotropy and LD evolve under selection (Barton 1990; Hansen et al. 2006; Melo and Marroig 2015; Pavlicev et al. 2011). Quantitative trait locus (QTL) mapping and genome wide association studies (GWAS) have begun to overcome this limitation, offering preliminary insights into the genetic architecture underlying covariation (especially for growth and morphological traits (Kenney-Hunt et al. 2008; Leamy et al. 2002; Porto et al. 2016; Wolf et al. 2005). In these studies, the link between pleiotropy and covariation has been made in a mostly qualitative manner using mapping locations, with effects estimated using ‘univariate’ models. Genomic regions that are mapped on to more than one trait are considered pleiotropic, and the observed covariation between these traits is related to this shared QTL. This method, along with trait specific effect estimates for each QTL, has proven very powerful for characterizing genetic architecture (Wagner and Zhang 2011; Wang et al. 2010). For example, several studies have pointed to a modular genotype-phenotype map, composed of predominantly local genetic effects, as the main driver of variational modularity in growth and morphological traits (Cheverud et al. 1996; Leamy et al. 2002; Mezey et al. 2000), in opposition to the equally plausible hypothesis of compensatory antagonistic and synergistic effects that can lead to variational modularity via hidden pleiotropy (Pavlicev and Hansen 2011), as was suggested in early quantitative genetics analysis (Cheverud et al. 1983).

Advances in statistical and computational methods have started to change the landscape of mapping approaches, with recent genome prediction and regularization methods opting for using all available markers in large prediction models, instead of mapping a small number of QTL of sufficiently large effects (de Los Campos et al. 2013; Meuwissen et al. 2001). In general, genome prediction methods perform well in predicting phenotypes, but at the cost of interpretability. Unlike mapping models, genome prediction distributes the genetic effects across all markers and does not identify specific QTL linked markers (this could be done after the model fitting by some variable selection procedure like in Piironen and Vehtari (2015) or Moser et al. (2015) but doing so is uncommon). GWAS and QTL mapping methods have also

advanced, with several mixed-model association methods allowing for the efficient analysis of a very large number of markers in structured populations (Lipka et al. 2012; Lippert et al. 2011; Zhou and Stephens 2012), all with good statistical performance (Eu-Ahsunthornwattana et al. 2014). However, these genome prediction and mixed-model association methods still deal poorly with multiple traits, and so we are limited to the traditional method of mapping traits separately and assessing pleiotropy *post-hoc*. These shortcomings may be overcome in the near future, with some promising methods being developed for efficient multivariate mapping (Hannah et al. 2018; Kemper et al. 2018; Pitchers et al. 2017). Multivariate mapping is fundamental for a qualitative study of pleiotropy, as it allows for detection of QTL with large effects overall but small effects on each individual trait under investigation. Multivariate mapping, when combined with quantitative genetics theory, also allows for a direct quantification of the effects of pleiotropic genetic effects on covariation, providing a much finer description of the genetic basis of covariation when compared to simple mapping of common QTL.

Here, we develop a multivariate mixed model framework to understand the genetic basis to patterns of covariation among growth traits in a mouse cross. Growth and size have long been ideal models for the study of genetic architecture, for several reasons. First, size is very amenable to artificial selection, having standing heritable variation and being easy to measure by several proxies, such as weight or length. Second, organismal growth unfolds through various stages, and these stages have different mechanisms and timings. For example, early quantitative genetics selection experiments showed that changes in cell number and cell size were somewhat independent mechanisms for changing the final size of the population under selection (Cheverud et al. 1983; Falconer et al. 1978; Leamy and Cheverud 1984; Riska et al. 1984). These stages of development can then be considered as several related traits interacting to form the final phenotype. Third, the covariation that exists between the different stages of growth (like early and late growth) provides an ideal system to investigate the relation between pleiotropy and covariation. We primarily focus on a QTL mapping model to study the

distribution of pleiotropic effects in order to link the pleiotropic effects of individual loci to the covariation between traits and to evolutionary change. We also adapt our mapping approach to fit a genome prediction model, allowing us to examine the differences and advantages of these alternative approaches. We apply these models to a population of mice derived from an intercross of mouse strains that diverged in size by univariate directional selection. By focusing on traits related to population divergence, we are able to place our genetic analyses in an evolutionary context. By using this explicit bottom-up approach to relate pleiotropy, selection and covariation, we ask: how malleable are patterns of covariation between traits? How much do they change under selection? Do patterns of pleiotropy align with selection or do they reflect developmental constraints? Can we use segregating variation to reconstruct the ancestral states of populations diverging by selection? All these questions help us come to a richer understanding of variation and of the evolutionary processes.

4.2 Methods

4.2.1 Study population

Our focal population is comprised of 1548 animals from the F₃ generations of an intercross between the inbred LG/J and SM/J strains of mice (for details see Cheverud et al. (1996); Wolf et al. (2008). These strains were derived independently by artificial selection for large (LG/J strain derived by Goodale (1938) or small (SM/J strain derived by MacArthur (1944) body weight at 60 days of age (see Chai (1956)). They differ by ca. 8.5 within-strain standard deviations in adult body weight (at 63 days of age) (Kramer et al. 1998). For simplicity, we refer to the lines as Large (LG/J) and Small (SM/J).

Details of the genotyping are provided by Wolf et al. (2008) and are only briefly outlined here. Each individual was genotyped at 353 SNP loci distributed across the 19 autosomes. Genotypes at each locus (*LL*, *LS* and *SS*, with the ‘L’ allele coming from Large and the ‘S’ allele coming from Small) were assigned additive (X_a) and dominance (X_d) genotypic index values,

where the values of X_a are $LL = +1$, LS and $SL = 0$, $SS = -1$, and for X_d are LS and $SL = 1$, LL and $SS = 0$ (see Wolf et al. 2008)

Animals were weighed weekly from one week of age. Our analyses focus on weight gained over each one week interval ('growth') from one week to seven weeks of age. These growth traits were calculated simply as the absolute difference in body weight between the weeks that define each time interval (e.g., $\text{growth}_{1,2} = \text{weight}_2 - \text{weight}_1$). See Vaughn et al. (1999) and Hager et al. (2009) for further details.

4.2.2 Phenotypic divergence

The vector of phenotypic divergence between founders was estimated as the difference between the means of the phenotypes of the founders. To estimate the direction of selection, we used a multiple regression of the growth traits in the F_3 with the target of selection, week 9 weight, and used the partial regression coefficients as the expected direction of the selection gradient (Lande and Arnold 1983). By multiplying this selection gradient to the observed G-matrix we also obtained an expected phenotypic divergence, which can be compared to the observed divergence. We also scaled the selection gradient so that the norm of the expected divergence vector is the same as the observed vector. This scaling is necessary because the magnitude of selection estimated by the multiple regression is too small to account for the many generations of selection. Using these multivariate vectors of selection and divergence, we measured the alignment of the estimated genetic effects (see below), phenotypic divergence and selection gradients. This allows us to characterize the genetic basis of the phenotypic divergence due to selection. Alignment between vectors was measured using vector correlations, that is, the cosine of the angle between the vectors being compared. We also investigate the relation between the norm of the pleiotropic effect vector and its alignment with the directions of selection and divergence.

4.2.3 Loci and alleles

We build our analysis on the classic quantitative genetic framework using a model of n biallelic loci that affect the value of t traits. At each j th locus we label alleles as L_j and S_j to indicate the allele originating from the Large and Small strains respectively. The frequencies of alleles are given by p_j for L_j and q_j for S_j . To build a multi-locus model we assemble genotypes from the four possible haplotypes at each pair of loci: $H_{L_jL_x}$, $H_{L_jS_x}$, $H_{S_jL_x}$, and $H_{S_jS_x}$ (hence the multi-locus genotype is assembled from pairwise combinations of loci). The frequencies of the four haplotypes at each locus pair depends on the frequencies of alleles at the two loci and the extent of linkage disequilibrium, such that: $H_{L_jL_x} = p_j p_x + \lambda_{jx}$, $H_{L_jS_x} = p_j q_x - \lambda_{jx}$, $H_{S_jL_x} = q_j p_x - \lambda_{jx}$, and $H_{S_jS_x} = q_j q_x + \lambda_{jx}$, where λ_{jx} is a measure of LD defined as: $\lambda_{jx} = H_{L_jL_x}H_{S_jS_x} - H_{L_jS_x}H_{S_jL_x}$.

4.2.4 Genetic effects

Genotypes at each locus, j , (listed as L_jL_j, L_jS_j, S_jS_j) were assigned additive ($X_j^a \in [1, 0, -1]$) and dominance ($X_j^d \in [0, 1]$) index values, such that:

$$\begin{bmatrix} \overline{L_jL_j} \\ \overline{L_jS_j} \\ \overline{S_jS_j} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} r_j \\ a_j \\ d_j \end{bmatrix} \quad (4.1)$$

where the overbar indicates the average phenotype associated with each genotype (i.e., the ‘genotypic value’ for each), which yields estimates of the additive and dominance genetic effects corresponding to the standard definition (Falconer and Mackay 1996)

$$\begin{bmatrix} r_j \\ a_j \\ d_j \end{bmatrix} = \begin{bmatrix} \frac{(L_jL_j + S_jS_j)}{2} \\ \frac{(L_jL_j - S_jS_j)}{2} \\ \overline{L_jS_j} - \frac{(L_jL_j + S_jS_j)}{2} \end{bmatrix} \quad (4.2)$$

The additive and dominance effects (i.e., genotypic values, defined in equation 2 above) of locus j were estimated as effects from a linear model:

$$E [z_i] = r_j + a_j X_{ij}^a + d_j X_{ij}^d + \varepsilon_{ij} \quad (4.3)$$

where z_i indicates the phenotypic value of individual i , r_j the reference point (representing the intercept), X_{ij}^a the additive and X_{ij}^d the dominance genotypic index values for individual i at locus j , and ε_{ij} the residual. Equation (3) can be extended to a multivariate form:

$$E [\mathbf{Z}_i] = \mathbf{r}_j + \mathbf{a}_j X_{ij}^a + \mathbf{d}_j X_{ij}^d + \boldsymbol{\varepsilon}_{ij} \quad (4.4)$$

Where Z_i is the vector (with length t) of traits measured for individual i . This model provides estimates of the vectors of additive ($\mathbf{a}_j = [a_{1(j)} \dots a_{t(j)}]$) and dominance ($\mathbf{d}_j = [d_{1(j)} \dots d_{t(j)}]$) effects, which summarize the pleiotropic effects of locus j across the t traits.

4.2.5 QTL mapping

We identified candidate QTL for the growth traits by fitting multivariate linear mixed models using dam as a random effect, and using separate fixed terms for the additive and dominance effects of the loci under consideration, as in equation (3) and (4). The simple family-level random effect controls for relatedness because all families in the F_3 are equally related. To estimate the QTL location, we used interval mapping models, by including flanking markers at various distances on either side of the focal marker (5, 10, 15 and 20 cM). Significance was assessed by dropping the focal marker and using a likelihood ratio test (LRT) with a Satterwhite correction. Models were fit in the R programming language using the lme4 package (Bates and Sarkar 2008), and the LRT was performed in the lmerTest package (Kuznetsova et al. 2017). We calculate chromosome-wise and genome-wise significance using a Bonferroni correction with the effective number of markers in each chromosome and in the whole genome. This method takes the correlation between markers (LD) into account when setting the overall significance threshold (Li and Ji 2005; Nyholt 2004). A list of markers and code for performing the mapping is available in the supporting information (SI).

Using the list of candidate markers, we then estimated the effects of each marker in all of the traits using a Bayesian multiple multivariate regression, again with family as a random effect. We used unit normal priors on the regression coefficients, centered Cauchy priors with unit scale on the variances and LKJ priors with scale 4 on the genetic and residual correlations between traits. This produces two vectors of effects for each marker on each trait, one for additive effects and one for dominance effects. We call these effect vectors pleiotropic vectors, as they measure the full pleiotropic effects of all the significant markers on the observed traits. This multiple regression was done in Stan (Carpenter et al. 2017) using custom code, also available in the SI¹.

4.2.6 Genome prediction

For the genome prediction, instead of running single marker models to select candidate markers, we ran one full model with all of the markers and used hierarchical shrinkage priors to produce regularized per-marker coefficients. Regularized coefficients are either heavily shrunk towards zero, indicating that the marker has no effect on a particular trait, or not shrunk, estimating the putative effect of that marker on a trait. This produces pleiotropic vectors for each marker in our model, but most of the coefficients in these pleiotropic vectors were close to zero. We implemented a custom version of the regularized horseshoe prior in Stan using the recommendations in Piironen and Vehtari (2017). This allowed us to include the family level random effect that accounts for relatedness and to partition the marker effect into an additive and a dominance components.

4.2.7 Estimation of quantitative genetic parameters

The genetic variance-covariance matrix was estimated from the null QTL mapping model (see above), where the same model was fitted without any marker information included. This provides a family-level estimate of the genetic covariance matrix based on the covariance

¹Code for fitting the models is available in <https://github.com/diogro/mouseGrowthQTLs>.

of full siblings. Because it is based on full siblings, it does not provide a direct estimate of the additive genetic variance-covariance matrix (i.e., the G-matrix). Rather, it is composed of one half of the additive genetic covariance matrix plus one quarter of the dominance genetic covariance matrix, plus any covariation due to shared environment within families. Therefore, we refer to this as the ‘full-sib’ genetic variance-covariance matrix.

4.2.8 Ancestral trait reconstruction

As a test of the quality of the pleiotropic genetic effect we estimated in the QTL mapping and genome prediction model, we used the estimated pleiotropic vectors to predict the phenotype in the ancestral founder populations of the Large and Small mice. We can predict the phenotype of the ancestral lines by asking what the phenotype of an individual of the F₃ generation would be if this individual had only Small or Large alleles. Both the QTL mapping and genome prediction models provide estimates of vector of additive effects of all loci on all traits. Each additive effect corresponds to half the difference between the average phenotypes of the alternative homozygotes. Therefore, the vector of additive effects can be multiplied by the index value of +1 to yield the estimated trait value of a Large allele homozygote across all loci, as a deviation from the F₃ mean (which represents the average reference point for the model). Likewise, multiplying the vector of additive effects by an index value of -1 yields an estimate of the phenotypic value of the Small allele homozygote across all loci (again, as a deviation from the mean).

$$E [Z_{LL}] = \mu + \sum_{j=1}^n a_j \quad (4.5)$$

and

$$E [Z_{SS}] = \mu + \sum_{j=1}^n -a_j \quad (4.6)$$

4.2.9 Estimation of genetic (co)variances

To separate heritable (additive) from non-heritable (dominance) genetic variation we first define the average effect of an allele substitution (α_j), which corresponds to the expected change in the value of each of the t traits resulting from replacing an S allele with an L allele. The vector α_j therefore summarizes the heritable (pleiotropic) effect of a locus since it reflects how changing an allele at a locus would, on average, change the phenotype of an individual. Although the genetic effects (a_j and d_j) are a property of the locus, the average effect of an allele substitution depends on the frequencies of alleles in a population:

$$\alpha_j = \mathbf{a}_j + [q_j - p_j] \circ \mathbf{d}_j \quad (4.7)$$

Where \circ indicates the Hadamard (element wise) product, which yields

$$\alpha_j = \begin{bmatrix} a_{1(j)} + d_{1(j)}(q_j - p_j) \\ \vdots \\ a_{t(j)} + d_{t(j)}(q_j - p_j) \end{bmatrix} \quad (4.8)$$

Equations (7) and (8) emphasize that the additive genetic variance contains two components, one caused by the additive effects and one caused by dominance effects.

Each j th locus contributes to the additive, $G_k = 2p_j q_j \alpha_{k(j)}^2$, and dominance, $D_k = (2p_j q_j d_{k(j)})^2$, genetic variances of trait k . Likewise, each locus contributes to the additive, $G_{kl} = 2p_j q_j \alpha_{j(k)} \alpha_{j(l)}$, and dominance, $D_{kl} = (2p_j q_j d_{k(j)} d_{l(j)})^2$, genetic covariances between traits k and l . The individual contributions of loci can be summed to yield the total additive and genetic (co)variances if loci are independent. However, when there is linkage disequilibrium there will be an additional component of (co)variation:

$$G_{kk} = \sum_{j=1}^n 2p_j q_j \alpha_{j(k)}^2 + \sum_{j=1}^n \sum_{x=1}^n \left[2\lambda_{jx} \alpha_{j(k)} \alpha_{x(k)} \right]_{j \neq x} \quad (4.9)$$

The second term on the RHS is summed over all pairs of loci not including a locus with

itself (hence the condition given that $j \neq x$), given that $\lambda_{jx} = \lambda_{xj}$. The first summation term on the RHS of equation (9) represents the contribution of individual loci to the total additive genetic variance, while the second term represents the additional variation caused by LD between loci. This latter term essentially represents the allelic covariance between loci, such that the total additive genetic variance is composed of a term arising from the effect of allelic variation (hence it being weighed by the squared average effect) and a term arising from the allelic covariance among loci (weighted by the product of the average effects of the alleles).

As for the off diagonal terms, the additive genetic covariance between traits k and l is given by:

$$G_{kl} = \sum_{j=1}^n 2p_j q_j \alpha_{j(k)} \alpha_{j(l)} + \sum_{j=1}^n \sum_{x=1}^n \left[2\lambda_{jx} \alpha_{j(k)} \alpha_{x(l)} \right]_{j \neq x} \quad (4.10)$$

The dominance genetic variance, like the additive genetic variance, contains a component arising from allelic variation and a component caused by linkage disequilibrium:

$$D_{kk} = \sum_{j=1}^n \left(2p_j q_j d_{j(k)} \right)^2 + \sum_{j=1}^n \sum_{x=1}^n \left[4\lambda_{jx}^2 d_{j(k)} d_{x(k)} \right]_{j \neq x} \quad (4.11)$$

Like for the dominance genetic covariance:

$$D_{kl} = \sum_{j=1}^t \left(2p_j q_j \right)^2 d_{j(k)} d_{j(l)} + \sum_{j=1}^n \sum_{x=1}^n \left[4\lambda_{jx}^2 d_{j(k)} d_{x(l)} \right]_{j \neq x} \quad (4.12)$$

From these definitions for the additive genetic variances and covariances, we can construct the additive genetic variance covariance matrix:

$$\mathbf{G} = \begin{bmatrix} G_{11} & \cdots & G_{1t} \\ \vdots & \ddots & \vdots \\ G_{t1} & \cdots & G_{tt} \end{bmatrix} \quad (4.13)$$

And the dominance genetic variance-covariance matrix:

$$\mathbf{D} = \begin{bmatrix} D_{11} & \cdots & D_{1t} \\ \vdots & \ddots & \vdots \\ D_{t1} & \cdots & D_{tt} \end{bmatrix} \quad (4.14)$$

Because the full-sib genetic variance-covariance matrix estimated using the mixed model in the F₃ population represents a mixture of additive and dominance components (see above), it is estimated as the sum $\frac{1}{2} \mathbf{G} + \frac{1}{4} \mathbf{D}$ from equations (13) and (14). Because the G matrix itself contains components arising from additive and dominance effects, we also calculated the additive genetic variance due to additive effects, G^a , and dominance effects, G^d by setting the additive (a_j) or dominance (d_j) effects to zero.

4.2.10 Matrix comparisons

To evaluate the quality of the matrix estimates, we compare the estimated covariance matrices using three complementary methods, which focus on different aspects of matrix structure. The Random Skewers method (Cheverud and Marroig 2007) summarizes the extent to which matrices are similar in the direction of their expected response to selection. This is done by multiplying the two matrices being compared by the same set of random selection gradients and taking the average of the vector correlations between the resulting expected response vectors. Significance of the Random Skewers comparison is calculated by comparing the observed vector correlation to the distribution of correlations from random vectors. The next method is a simple element wise correlation of matrix elements, which can be used in correlation matrices as a measure of the similarity in the pattern of association. Significance of the matrix correlation is done using the Mantel permutation method, with takes the non-independence of the individual elements in the matrix into account. The Krzanowski correlation measures the congruence of the spaces spanned by the first half of the eigenvectors of the matrices being compared. We do not calculate a significance in relation to the Krzanowski method. See (Melo et al. 2015) for details on all these comparison methods.

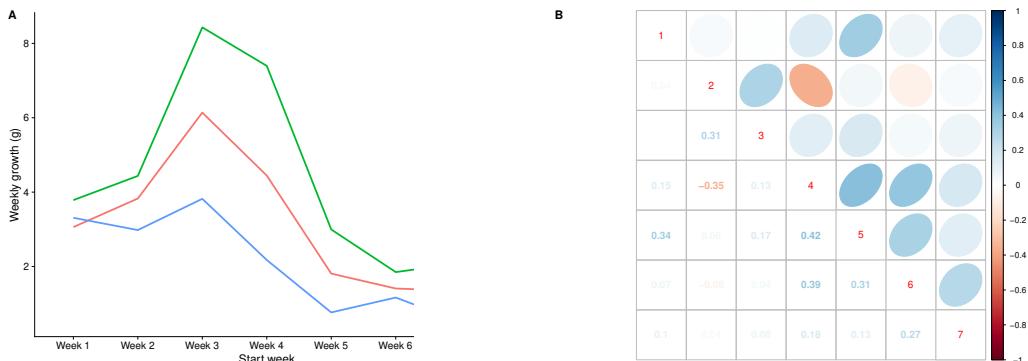


Figure 4.1: Growth curves. (A) Weekly growth for the founders (Large shown in the top line (green), and Small in the lower line (blue)) and the F₃ generations (middle line, red). In most growth periods, the F₃ generation is between the two founders. (B) Genetic correlations from the full-sib genetic matrix in the F₃ generation. Smaller correlations are more transparent and related ellipses less eccentric, larger correlations are more opaque and ellipses more eccentric. Positive correlations in blue and negative correlations in red.

4.3 Results

4.3.1 Growth curves

Growth curves for the parental and F₃ generation suggest an almost completely additive behavior of the weekly growths, with the F₃ generation being intermediate between the two founders for most traits, except for the first week, in which the F₃ generation is smaller than both founders (Fig. 4.1A). Genetic correlations between growth periods are generally positive, except for a negative correlation of -0.36 between growth in weeks 2 and 4. Larger correlations are present in later growth (around 0.3 to 0.4 in adjacent weeks and between weeks 4 and 6). Early growth shows a positive correlation between weeks 2 and 3. Most correlations between early and late growth are small, except for a .34 correlation between weeks 1 and 5. There is also a small negative correlation between weeks 2 and 6 (Fig. 4.1B). In summary, during early growth week 1 is mostly independent, weeks 2 and 3 are positively correlated; and during late growth and weeks 4-7 are positively correlated and somewhat independent of early growth.

4.3.2 Selection and divergence

The observed phenotypic divergence, the estimated selection gradient and the expected phenotypic divergence given this gradient are shown in Table 4.1 and Fig. S4.1. Vector correlation between observed and expected phenotypic divergence is high ($r = 0.93$), indicating that the observed divergence is compatible with the expected from selection and covariation in the F_3 .

Table 4.1: Vectors of phenotypic divergence, estimated selection gradient in the F_3 and expected divergence given the estimated selection.

Week interval	Observed divergence (g) Δz	Estimated selection gradient (scaled)	Expected phenotypic divergence
Week 1 - 2	0.475	3.336	1.221
Week 2 - 3	1.455	7.377	2.823
Week 3 - 4	4.610	7.666	3.986
Week 4 - 5	5.220	6.146	3.947
Week 5 - 6	2.230	6.606	3.178
Week 6 - 7	0.685	5.982	2.348
Week 7 - 8	1.575	4.323	1.494

4.3.3 QTL mapping

We identified 32 putative QTL loci using our multivariate regression model with flanking markers. Position of the chosen markers is shown in Fig. 4.2. Pleiotropic effect vectors were simultaneously estimated for all chosen markers and are shown in Fig. 4.3. All markers show some degree of pleiotropy, affecting as few as two and as many as all 7 traits (Fig. S4.2). Additive effects are, in general, larger than dominance effects, and the total size of the effect vector was not related to the level of pleiotropy. A principal component analysis (PCA) of the marker effects reveals that the first two principal components of the additive effects (responsible for 71% of the variation) correspond to the early and late growth phase, suggesting two somewhat independent directions of variation in genetic effects. No such separation is visible in the dominance effects, but we can see a split in the loadings of PC1, with early

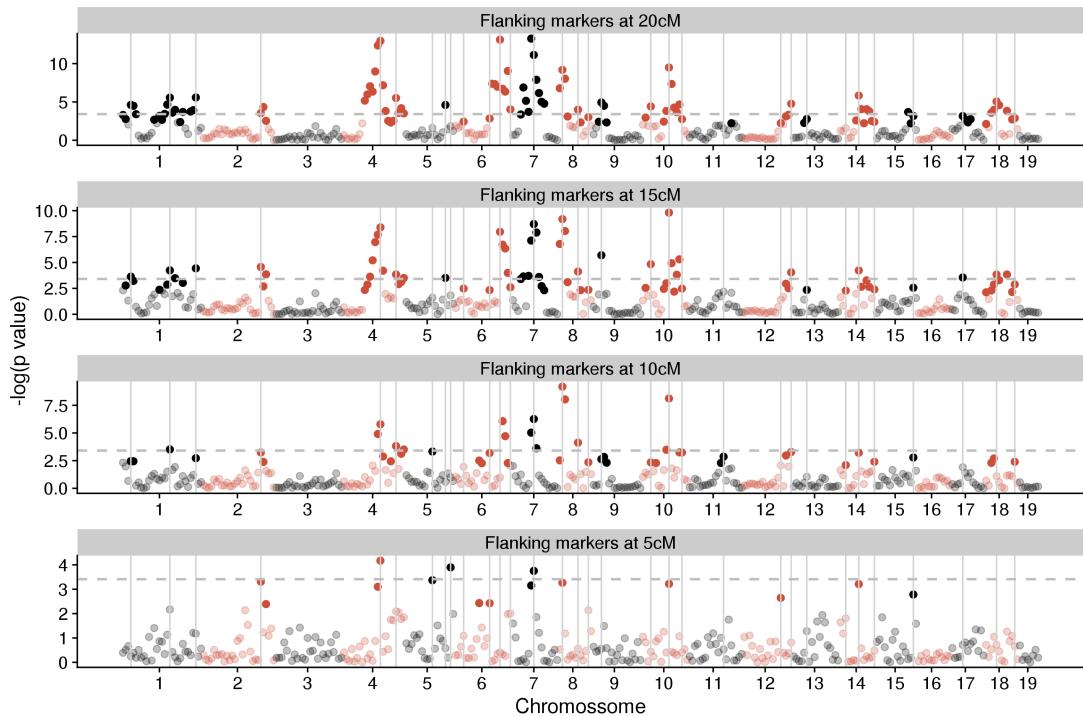


Figure 4.2: Identified makers using interval mapping with various flanking marker distances. Chosen markers are shown as gray vertical lines. Significant markers at the chromosome levels are shown in full color, non significant markers at the chromosome level are translucent, the dashed line marks the whole genome significance threshold. Chromosomes are shown in alternating colors.

and late traits taking on opposite signs (Fig. 4.3C and D). In the dominance effects, the first two PCs account for 56% of the variation. When comparing the direction of the pleiotropic vectors with the direction of phenotypic divergence, the mean additive vector is very aligned with divergence (vector correlation of 0.96), whereas the mean dominance vector is unaligned (vector correlation of 0.11). Additionally, we see a significant relation between the norm of the individual additive vectors and their alignment with divergence and the selection gradient: larger pleiotropic vectors being more aligned with both (alignment with divergence, slope = 1.73, $p = 0.002$; alignment with selection gradient, slope = 1.69, $p = 0.005$, Fig. 4.4A and C). No such relation is present in the dominance vectors (alignment with divergence, slope = -0.40, $p = 0.67$; Alignment with selection gradient, slope = -0.36, $p = 0.66$, Fig. 4.4B and D).

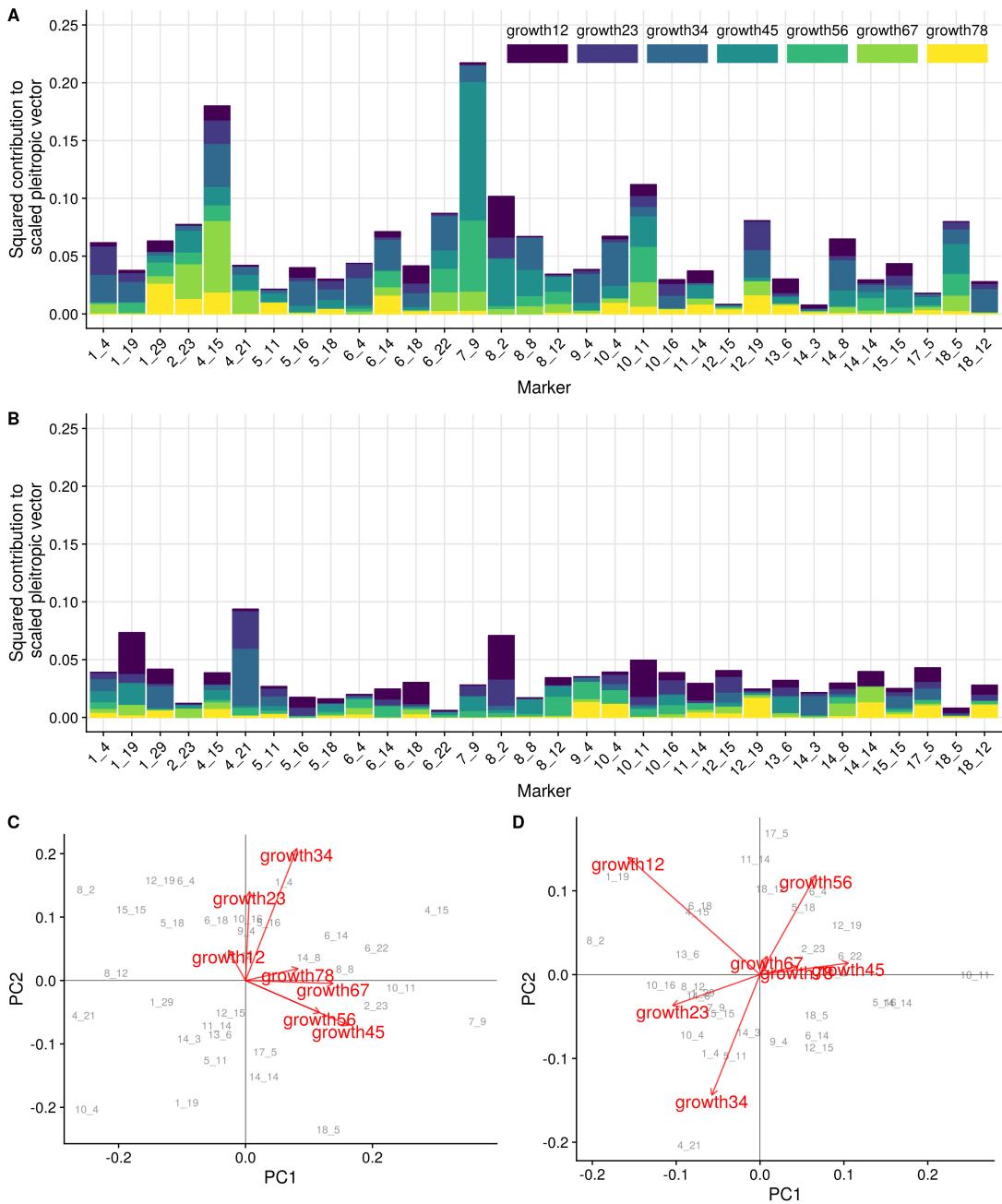


Figure 4.3: Pleiotropic effects of identified markers. (A) additive and (B) dominance contributions of the trait components to the final length of the pleiotropic vector. All trait contributions are scaled to trait standard deviation and are comparable. (C) additive and (D) (dominance): PCA of marker effects, arrows represent trait loadings in PC 1 and 2, marker IDs in grey are marker scores in PC 1 and 2. Markers are coded as chromosome and marker within chromosome, see SI for genomic position.

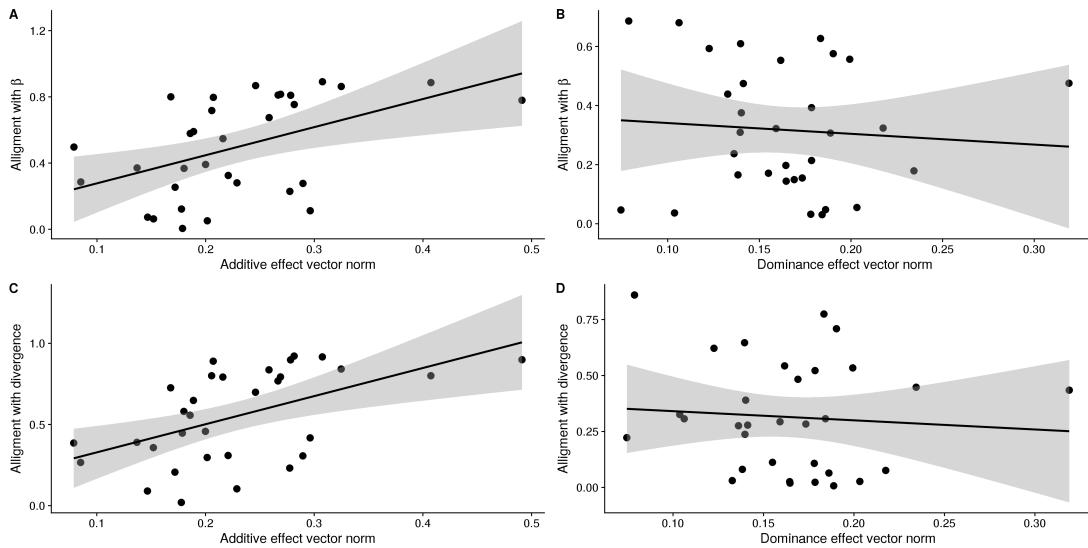


Figure 4.4: Size and alignment for chosen markers. Pleiotropic effect vector alignment with selection and divergence between founders. Panels A and B show the relation between pleiotropic effect vector norm and alignment to estimated selection gradient (β), while panels C and D show the relation between pleiotropic effect vector norm and alignment to phenotypic divergence. A) and C) additive effects; B) and D) dominance effects.

4.3.4 Genome Prediction

Genome prediction produces pleiotropic effect vectors for all markers (Fig. 4.5). We again see a widespread pattern of pleiotropy, but less so than in the mapped effects, as several of the large effect vectors have effects in only one or two traits. This is especially evident in the dominance effects. The PCA plot of marker effects confirms this, as the first two PCs are not related to early and late growth, as in the mapped markers, but combinations of single trait effects. We can also see the pattern of linkage affecting the pleiotropic vectors, as larger effects are often clustered and similar, suggesting that the regularization shrinkage prior was unable to pick a single marker as the best position for the effect, spreading the putative QTL over several neighboring markers (this is a known limitation of this type of sparse regression, see Piironen and Vehtari (2017)). We can also see the pattern that larger additive effect vectors are more aligned with phenotypic divergence. Small effects, which were shrunk toward zero by the horseshoe prior, are essentially pointing in random directions, while larger additive vectors

are more aligned with divergence and selection (alignment with divergence, slope = 2.42, $p < 0.001$; alignment with selection gradient, slope = 1.75, $p < 0.001$, Fig. 4.6A and C). Dominance vectors again don't have any pattern between magnitude and alignment (alignment with divergence, slope = -2.01, $p = 0.09$; alignment with selection gradient, slope = -2.14, $p = 0.08$, Fig. 4.6B and D).

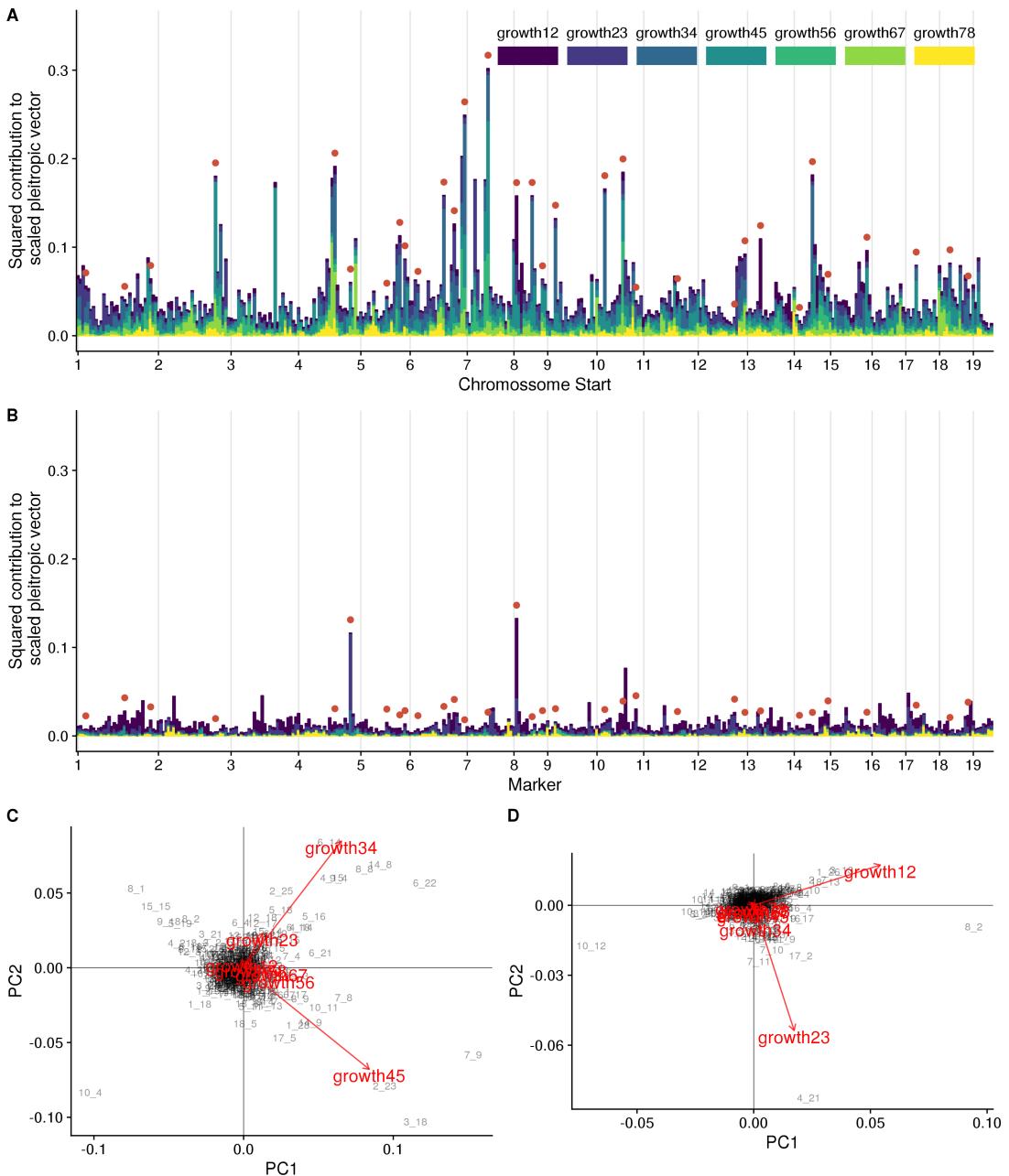


Figure 4.5: Regularized pleiotropic effects of all markers. (A) additive and (B) dominance contributions of the trait components to the final length of the pleiotropic vector. Significant markers in the QTL mapping are marked by the red dots. All trait contributions are scaled to trait standard deviation and are comparable. (C) additive and (D) dominance PCA of marker effects, arrows represent trait loadings in PC1 and PC2, marker IDs in gray are marker scores in PC 1 and 2.

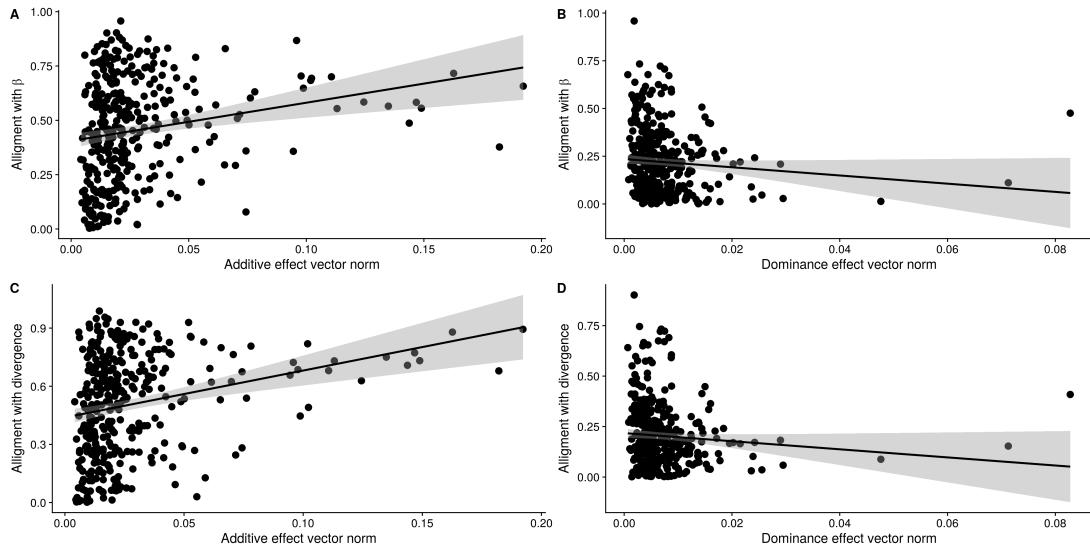


Figure 4.6: Regularized pleiotropic effect vector alignment with selection gradient and divergence between founders. Panels A and B show the relation between regularized pleiotropic effect vector norm and alignment to estimated selection gradient (β), while panels C and D show the relation between pleiotropic effect vector norm and alignment to phenotypic divergence. A) and C) additive effects; B) and D) dominance effects.

4.3.5 Ancestral predictions

Both regression models do well at predicting the phenotypes of the founder strains using only genetic effects estimated from the F_3 generation (Fig. 4.7). The genome prediction method is slightly better on the average prediction, but most ancestral growth periods are inside the posterior distribution (grey lines) for both models. First week of growth is somewhat anomalous in that the F_3 generation is not intermediate to the two founders, possibly due to environmental effects, and so the prediction is poor. Growth in week 4 in the Large strain is larger than predicted from the F_3 genetic effects, suggesting either non-detected effects in the F_3 or some other non-additive genetic or environmental effects. The same applies to the week 7 growth, where both Large and Small strains are more different from the F_3 than expected from the F_3 additive effects.

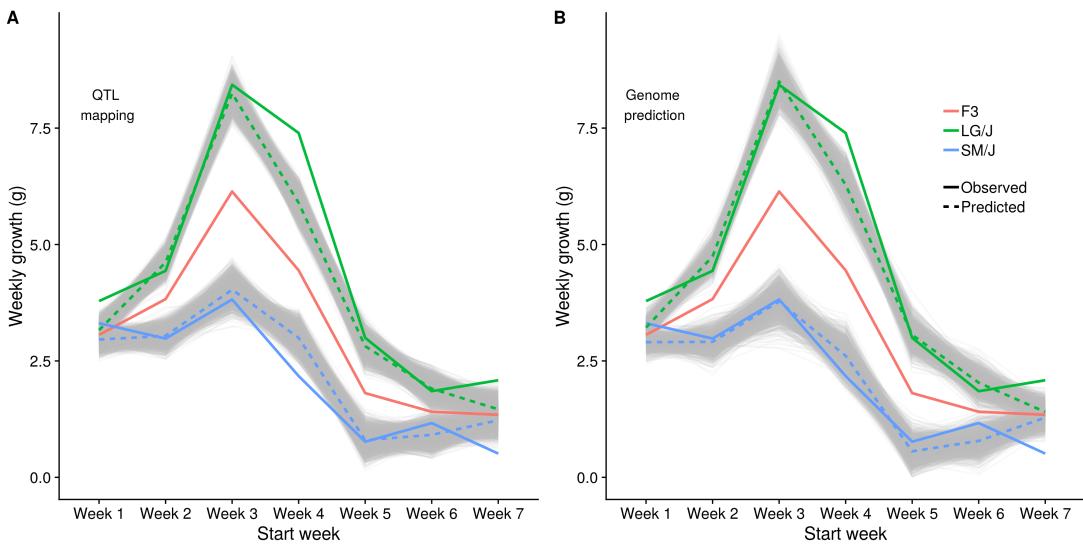


Figure 4.7: Ancestral predictions from additive effects. Predictions of the ancestral growth curves using additive effects estimated in the F_3 generation. Solid lines are the observed growth curves, dashed lines the predicted growth curves from the two models. (A) QTL mapping of significant markers, (B) Genome prediction using all markers. Grey lines represent the posterior distribution of ancestral predictions derived from the Bayesian effect estimates.

4.3.6 Covariance matrix predictions

Genetic correlation matrices estimated from the pleiotropic effects from the mapped markers are broadly similar to the family level full-sib genetic matrix estimated from the mixed model fit. We can see the same strong correlation between late growth, and the positive correlation between weeks 2 and 3. The negative correlation between weeks 2 and 4 is present, but much smaller in magnitude in the QTL estimated matrix. The smallest correlations are between the early and late traits. Regressing the full-sib genetic matrix onto the genetic covariances predicted from the mapped markers (given by the sum of half the additive genetic matrix with one quarter of the dominance genetic matrix) reveals that the observed covariances are in general much larger than the predicted ones, but the pattern of covariances is similar (intercept = -0.01, slope = 2.43, $p < 0.001$). The outliers in the regression are the negative covariance between weeks 2 and 4 and the much larger observed variance in week 4. This is not surprising, since the full-sib genetic includes other sources of covariation, like maternal

and common environmental effects. The genome prediction fared much worse, predicting variances and covariances close to zero for almost all traits (see Fig. S4.3). Matrix comparisons can be seen in Table 4.2. The most similar matrices are the QTL mapping marker genetic matrix, followed by the QTL mapping marker additive genetic matrix. Both show high similarities in Random Skewers, Mantel matrix correlation, and a high proportion of shared subspace. The dominance matrix shows a relatively low matrix correlation and the lowest Krzanowski shared sub-space correlation, suggesting it is different in structure to the full-sib genetic matrix. Genome prediction matrices have very low, non-significant Mantel correlation values, reflecting the poor estimate of the G-matrix correlations. Random Skewers comparisons are all relatively high and significant, with the exception of the genome prediction dominance matrix. This can be due to a relatively similar first principal component in all matrices.

Table 4.2: Matrix comparisons of the QTL mapping and Genome prediction marker based matrices with the estimated full-sib genetic via Random Skewers, Mantel correlation and Krzanowski shared subspace correlation.

Matrix	Similarity to Family full-sib genetic matrix		
	Random Skewers	Mantel correlation	Krzanowski correlation
QTL-mapping marker additive genetic matrix	0.872 (p < 0.001)	0.69 (p < 0.001)	0.86
QTL-mapping marker dominance genetic matrix	0.85 (p = 0.005)	0.49 (p = 0.01)	0.62
QTL-mapping marker genetic matrix ($\frac{1}{2} G + \frac{1}{4} D$)	0.89 (p < 0.001)	0.70 (p = 0.002)	0.86
Genome prediction marker additive genetic matrix	0.82 (p = 0.007)	0.09 (p = 0.28)	0.78
Genome prediction marker dominance genetic matrix	0.6 (p = 0.057)	0.20 (p = 0.16)	0.40
Genome prediction marker genetic matrix ($\frac{1}{2} G + \frac{1}{4} D$)	0.83 (p = 0.002)	0.12 (p = 0.29)	0.77

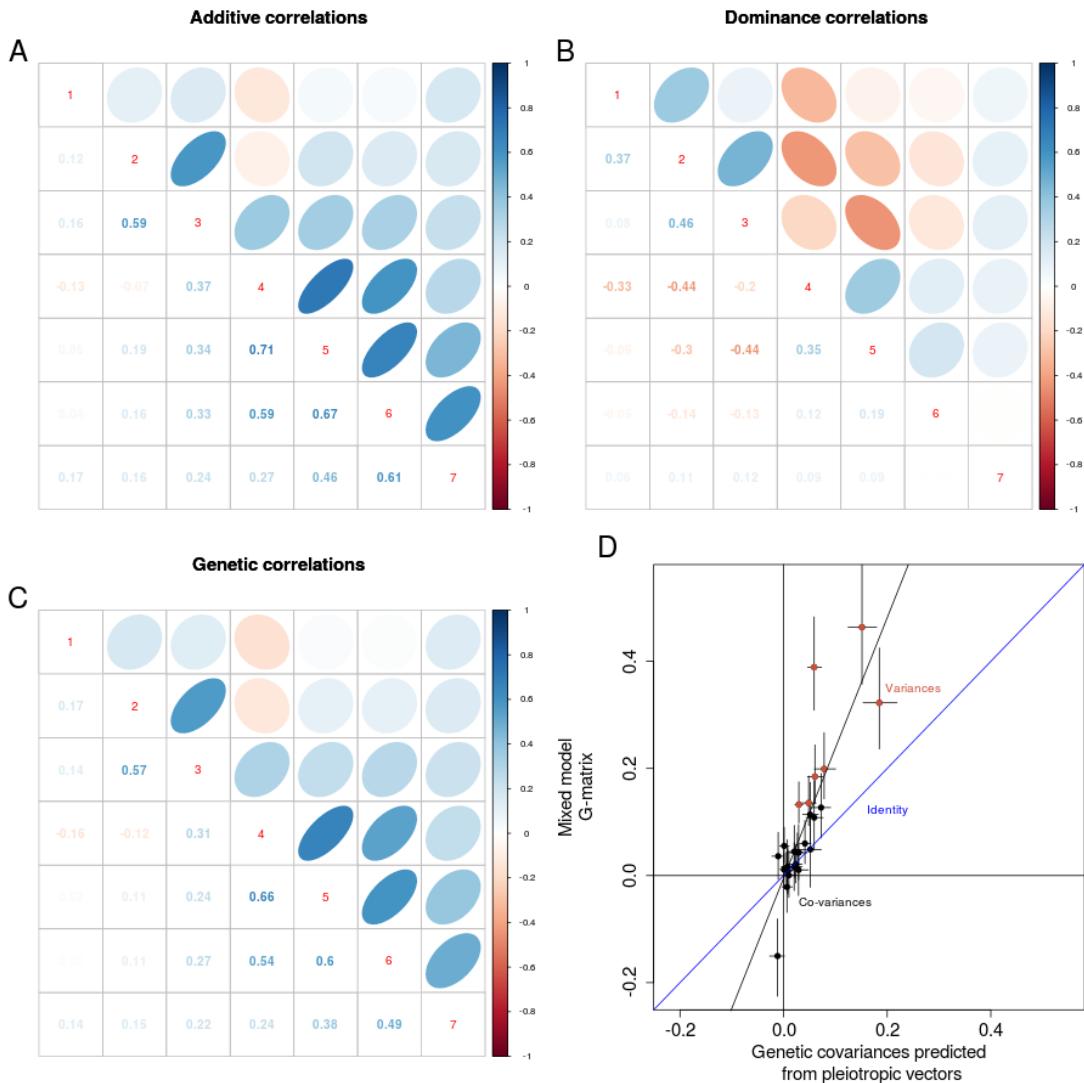


Figure 4.8: Additive, dominance and genetic matrices. Estimated correlation matrices from QTL mapping marker effects. (A) additive, \mathbf{G} , (B) dominance, \mathbf{D} (C) Genetic, $\frac{1}{2} \mathbf{G} + \frac{1}{4} \mathbf{D}$. (D) Regression of genetic variances and covariances estimated from full-sib mixed model and estimated from markers. Error bars represent 95% posterior credibility intervals. Marker-estimated covariances are about half of observed covariances.

4.4 Discussion

The interaction between selection and multivariate covariation is a central part of our understanding of evolution. Even simple selection regimes can produce complex multivariate responses due to cascading developmental effects and genetic constraints. Here, we use a cross between two artificially selected lines of mice to investigate the genetic architecture of the multivariate response due to artificial selection. The target of selection in both lines was individual final weight, in opposite directions, and we use the various phases of growth as a model of a multivariate trait altered by selection. Using multivariate QTL mapping and genomic regression, we were able to map loci involved in the variation of growth and to simultaneously estimate vectors of genetic effects responsible for the variation in growth. Combining these effect vectors with quantitative genetics theory, we were able to directly link the pattern of pleiotropy and the genetic covariation of these traits.

As expected by the behavior of the phenotypes in the cross (Cheverud et al. 1996), most of the divergence between Large and Small is due to additive effects. The mean additive effect vector is practically collinear with the direction of divergence between lines, and we see a relation between the total size of the additive effects and the alignment with selection and divergence. This relation between alignment and size reflects the effect of selection removing large effects in other directions, that is, the larger the pleiotropic effect the more it must conform to the direction of selection in order to be maintained, while smaller effects can be less aligned with selection and still contribute to the divergence between lines. This clear alignment is absent from the dominance effect vectors, as the mean dominance vector has low correlation with the phenotypic divergence and the direction of selection, and the size of the individual marker effects also has no bearing on their alignment to divergence and selection. Dominance vectors are not expected to contribute to the phenotypic divergence, since they are an interaction effect between alleles from the two founder lines, and so are only present in the crosses. These relations between effect size and alignment are present

in both QTL mapping and genome prediction estimates of the additive and dominance effect vectors, but in the genome prediction the relations are much less clear when we look at the very small vectors. These small effect vectors are distributed in all directions but have very small norms (which is a measure of the overall effect of the locus across traits). Presumably, this is indicative that they have negligible effect on the phenotype and are being shrunk to zero by the sparse regression priors, and so the individual vector directions are essentially random.

Ancestral predictions using QTL mapping is surprisingly accurate, and most of the ancestral traits are within the posterior distribution of ancestral estimates, even though we are using only a small number of large effect QTL in the prediction model. The genome prediction analysis using sparse regression, on the other hand, is potentially including a number of small effects that do not meet a significance threshold, and so are excluded from the QTL mapping analysis. The inclusion of these many small effects have been proposed as a solution to the missing heritability problem (Bloom et al. 2013; Boyle et al. 2017), and modern sparse regression and genomic prediction methods provide a promising framework for working with high density marker data sets (Pong-Wong 2014). Indeed, the sparse regression produces very good out-of-sample predictive performance when used to predict the phenotypes of the founder lines (Fig. 4.7). Mapping could also be done in this sparse regression framework using variable selection, and the model displays reasonable agreement with the QTL mapping, producing large effect estimates that are close to the detected QTL (Fig. 4.5). Furthermore, our analysis was done in standard generic Bayesian model fitting software (admittedly this was only possible since our marker data set is relatively low density, but further advances in general statistical software should make using similar approaches in larger datasets possible). QTL mapping, on the other hand, provides us with a small set of interpretable pleiotropic vectors, which capture the modular aspects of early and late development, and can be used to understand the pattern of covariation that we see in the G-matrix. Although the inclusion of all the marker helps with the ancestral prediction and uses effects that are ignored by the

QTL mapping, using all of the markers for the estimation of expected covariances seems to be much more susceptible to noise introduced by small effect markers than the mean ancestral predictions, and the marker estimated variances and covariances using genome prediction are all close to zero. Some sort of variable selection would be necessary to make this estimate reliable using the genome prediction estimates. We do not pursue this further, but presumably even gradual removal of small effects would improve this estimate.

Using the pleiotropic effect vectors estimated in the QTL mapping analysis and quantitative genetics theory (Kelly 2009), we were able to construct expected additive and dominance genetic covariance matrices. The additive marker matrix is broadly similar to the observed full-sib genetic matrix, with strong positive correlations between the late traits, a strong correlation between weeks 2 and 3, and weaker correlations between early and late traits. The dominance matrix is different, with weak positive correlations within early and late traits and negative correlations between them. This clean separation between additive and dominance components of the G-matrix is difficult to achieve using only breeding experiments, and underscores how different these genetic effects can potentially be. The difference in the patterns of additive and dominance covariation suggests that these patterns have considerable latitude to vary and do not arise from a fundamental property of the system. If all gene effects on growth were constrained by some set of developmental pathways, we would expect all sources of genetic covariation to share a similar pattern, reflecting the developmental constraints. (For example, a trade-off could restrict a locus with positive effects on trait A to have negative effects on trait B regardless of the effects being additive or dominant). The genetic matrix, composed of a one half the additive matrix plus one fourth the dominance matrix is similar to the full-sib matrix, but with smaller variances throughout. The negative correlations between weeks 2 and 4 is present, but not significant, in the marker estimated matrix (Fig. 4.8C). Given that our maker based estimates does not include several components that are expected to contribute to the genetic matrix, like shared environment and maternal effects, it is not surprising that the marker estimated variances are smaller than the observed genetic covariances. The

smaller variances and covariances in the QTL mapping maker based genetic matrix can also be attributed to the inclusion of only part of the direct genetic effects, since only loci with larger effects are used. Nevertheless, the general structure of the full-sib genetic matrix is captured by the expected covariance due to pleiotropy and linkage of the mapped loci, as we can see in the high values of matrix similarity and in Fig. 4.8. The success in predicting the pattern covariation directly from pleiotropic effects underscores the importance of pleiotropy in determining genetic covariation, and suggests that a relatively small number of medium and large effect loci can be responsible for a large portion of genetic constraints. Another possibility is that the distribution of pleiotropic effects is shared between small and large effects, either due to mutation, selection bias, or both. This shared distribution would explain why the general pattern of covariation, but not the total amount, is successfully predicted from a small number of markers.

The distribution of pleiotropic effects in the QTL mapping analysis offers some insights into the genetic architecture of growth. First, we see that the full distribution of additive pleiotropic effects spans a modular variational space, with independent principal components aligned with the two stages of growth, early and late. This is somewhat unexpected given that the vector of selection on growth was in the direction of coordinated change in all phases of growth (Table 4.1), either increasing or decreasing the target of selection, week 9 weight. Given this target of selection, we would expect the distribution of the additive effects responsible for the divergence between large and small to be wholly aligned with the coordinated change of all growth phases, either increasing or decreasing all phases of growth. Indeed, on average, the additive effects are aligned with divergence, and large effects more so, but we still maintain a number of pleiotropic vectors with antagonistic effects in both phases, either increasing early traits and decreasing late traits or *vice-versa* (markers on the top-left and bottom-right quadrant of Fig. 4.3C). The maintenance of this modular variation in pleiotropic effects could be associated with developmental or mutational constraints on the additive genetic effects. But, if this were the case, we might expect the dominance effects, which are not shaped by selective

history of the founders, to have a similar modular pattern. While the dominance effects PCA does not show the same clear early-late separation in orthogonal directions that we see in the additive effects, the dominance genetic matrix has a different distinction between early and late, with positive correlations within each phase and negative correlations between. So perhaps the modular aspect of the genetic effects can manifest in different ways. Additionally, the variational modularity between early and late growth that we see in the G-matrix is not only due to markers having modular effects, restricted to one stage of growth or another (markers close to the x and y axis in Fig. 4.3C), but is also due to a combination of markers that have general effects in both stages (markers along the main diagonal, in top-right and bottom-left quadrants in Fig. 4.3C) or the previously mentioned markers with antagonist effect in both stages. This reinforces the idea that there are several genotype-phenotype maps that can generate a modular covariance matrix (Pavlicev and Hansen 2011). However, several studies have found very low levels of antagonistic pleiotropy and many more modular pleiotropy in morphological traits (Kenney-Hunt et al. 2008; Leamy et al. 2002, 1999), suggesting that perhaps the specific genetic architecture underlying modularity could vary depending on the type of trait and on its evolutionary history.

The explicit link between genetic effects and covariation is a natural way to study multivariate evolution, but still rare in the literature (Kelly 2009). Using this approach, we were able to decompose the full-sib genetic matrix into its additive and dominance components. These components show different patterns of covariation, a consequence of the differences in the additive and dominance distributions of pleiotropic effects. Although both classes of genetic effects show some signal of the division between early and late growth, the modular pattern is much more obvious in the additive effects. The full-sib covariance matrix, a common proxy for the additive genetic covariance matrix, is similar to the purely additive genetic matrix, but could differ more depending on the dominance component. Furthermore, we were able to accurately reconstruct the ancestral states in the founders using only the effects estimated in the F₃ population, and this prediction was improved using all the markers in a sparse genome prediction model.

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Supporting Information

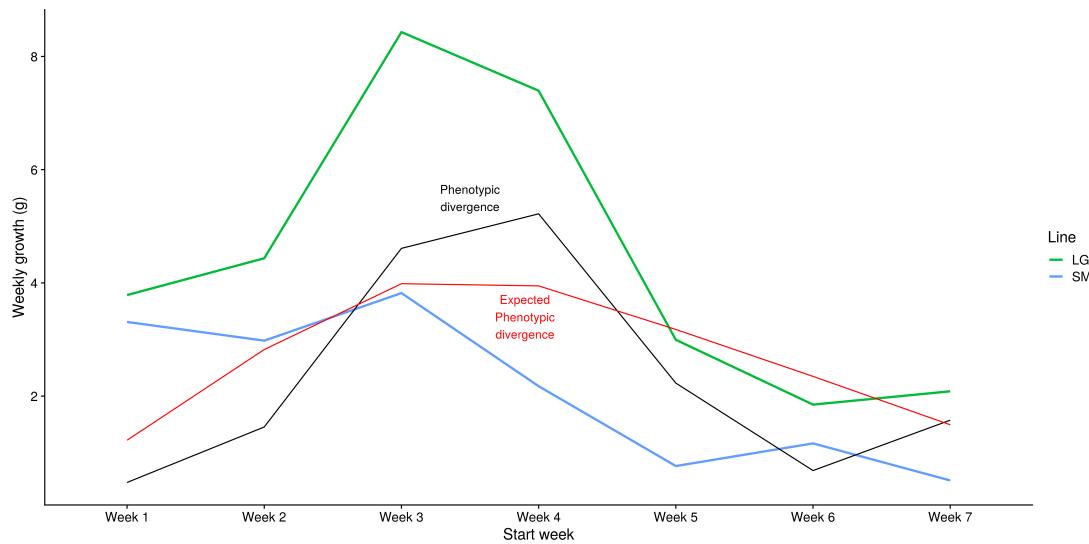


Figure S4.1: Observed and expected phenotypic divergence in the parental lines. Expected divergence is estimated using the F_3 generation to estimate a selection gradient on growth due to selection on week 9 weight, and the expected divergence is estimated by multiplying this selection gradient by the growth full-sib genetic matrix. The resulting expected divergence is scaled to have the same magnitude as the observed divergence.

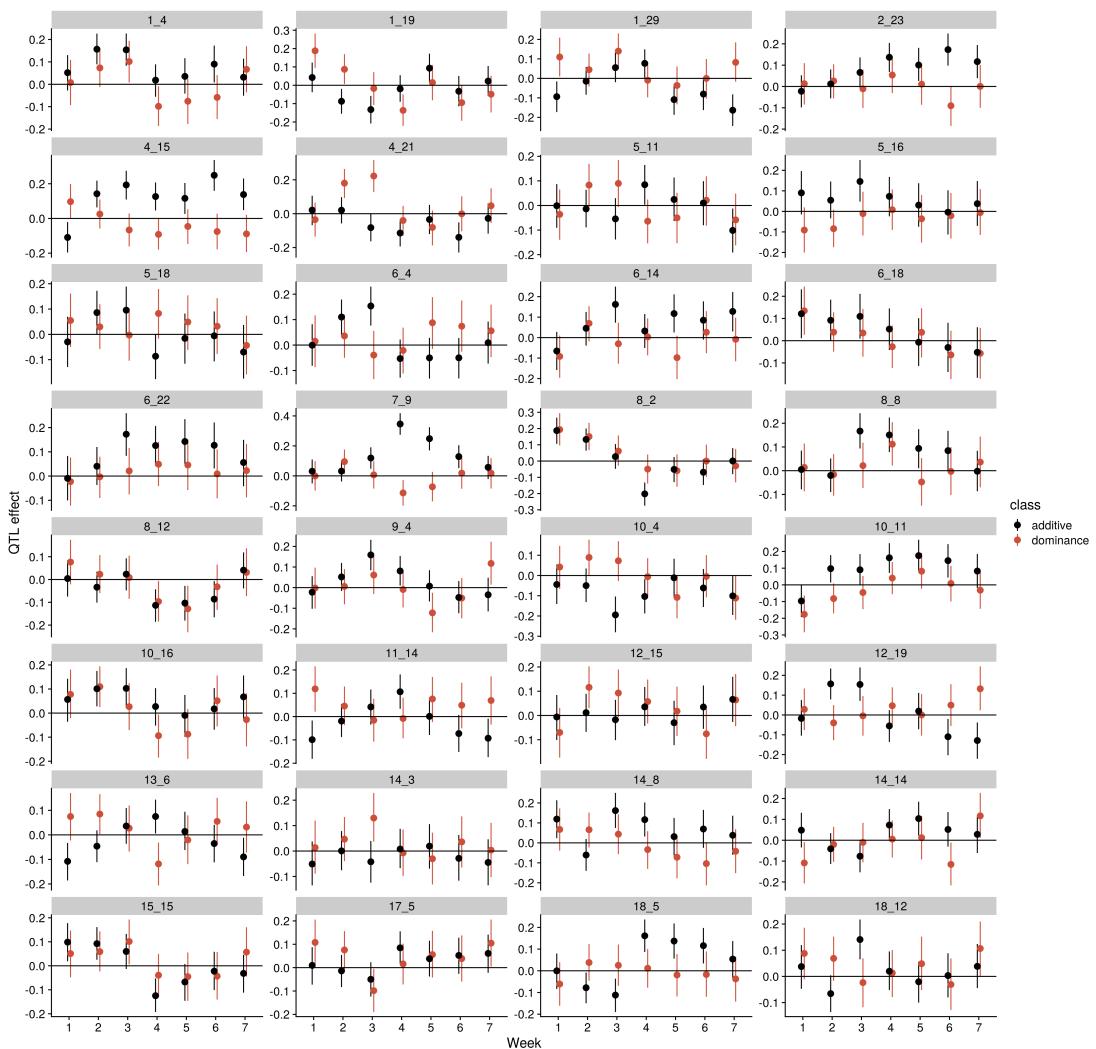


Figure S4.2: Pleiotropic effects of all significant loci in the QTL mapping model.

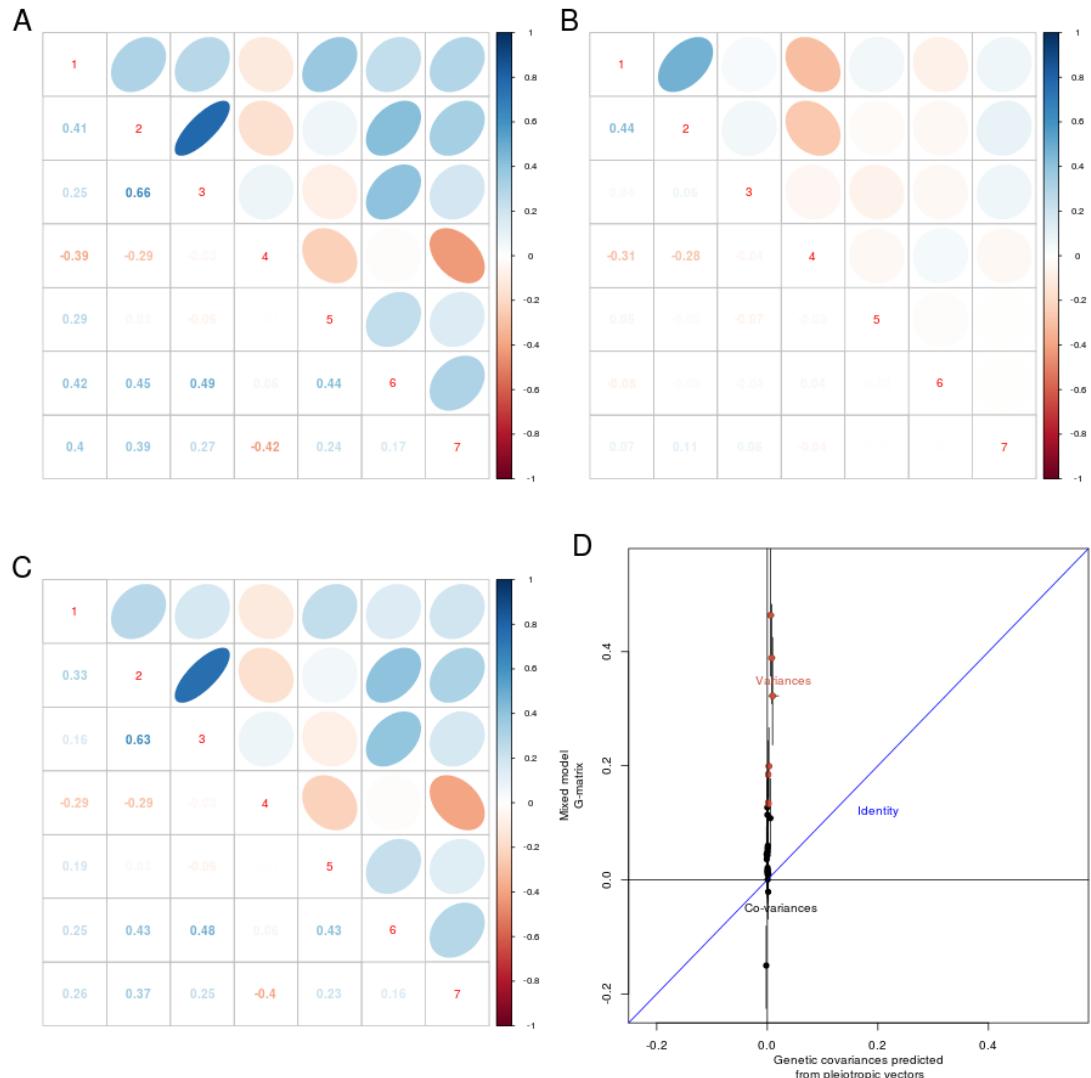


Figure S4.3: Additive, dominance and genetic matrices. Estimated correlation matrices from genome prediction marker effects. (A) additive, (B) dominance (C) Genetic, $\frac{1}{2}$ additive + $\frac{1}{4}$ dominance . (D) Regression of genetic variances and covariances estimated from full-sib mixed model and estimated from markers. Error bars represent 95% posterior credibility intervals. All estimated variances are essentially zero, and correlation matrices are different from the population full-sib genetic matrix.

Chapter 5

Genetic Architecture and the Evolution of Variational Modularity

Diogo Melo, Eleanor O'Brien, John Hunt, Jason Wolf, Gabriel Marroig

Abstract

Genetic architecture interacts with the evolutionary processes and is a key factor in determining short and long-term evolution. For a set of traits, the evolutionary response to selection and expected outcome of multivariate drift depend on the pattern of genetic covariation. Ultimately, genetic covariation is determined by genetic architecture and allelic variation, and we hope to understand how genetic architecture can influence evolution by studying covariation. However, it is not clear how closely additive genetic covariation reflects the underlying genotype-phenotype map, as several GP-maps can lead to the same pattern of covariation. We use an explicit individual-based simulation approach to investigate how genetic effects evolve under different selection regimes, and how these evolving genetic effects are reflected in the pattern of covariation. We confront these computational results with multivariate mapping of genetic effects in an experimental population of mice composed from lineages derived by artificial selection for changes in growth trajectories. We estimate the multivariate effect of loci on growth and use these effect ‘vectors’ to examine how pleiotropy in the genotype-phenotype map determines genetic covariance structure. We show that under simulations and in real populations the covariance pattern is determined by a complex combination of different types of genetic effects that interact to determine the pattern of covariation.

Keywords: G-matrix; QTL mapping; genome prediction; genetic architecture

5.1 Introduction

Heritable genetic variation determines a trait's evolvability, defined as the ability to respond to selection. In order to efficiently respond to selection, traits must have enough genetic variation to change under selection, and this variation must be somewhat independent from the rest of the organism (Lewontin 1979). Genetic correlations captures the pattern of genetic dependency between traits (Lande 1979; Lande and Arnold 1983) and a modular variational pattern, in which functionally related traits are more correlated with each other while being less correlated with other traits, provides an organization that permits groups of traits to evolve in a coordinated fashion while not disturbing other traits (Cheverud 1996; Melo et al. 2016; Olson and Miller 1958; Wagner et al. 2007).

The genetic association between traits is largely determined by the pleiotropic relations between alleles and phenotypes, and shared development, both of which are summarized in the genotype-phenotype (GP) map . If pleiotropic effects are mostly synergistic with respect to phenotypes, affecting trait means in the same direction, traits that share more pleiotropic effects will be more correlated, while traits that share few effects will be less correlated. This leads to the hypothesis that the variational modularity we observe in the population is generated by a particular type of GP map, in which the genetic effects themselves are modular and restricted to a limited number of related traits (Wagner and Altenberg 1996). Indeed, some degree of modularity in the organization of genetic effects has often been shown to underlie modular variation (Cheverud et al. 1997; Kenney-Hunt et al. 2008; Leamy et al. 1999; Mezey et al. 2000; Porto et al. 2016). However, the relation between pleiotropy and covariation is complicated by a many-to-one mapping between pleiotropy and variational modularity, as many different GP-maps can generate the same covariation pattern (Mitteroecker 2009). In particular, a modular GP map is not a prerequisite for variational modularity, as pleiotropic effects can also affect two traits or modules in opposite directions, creating antagonistic pleiotropy. Antagonistic pleiotropy can generate variational modularity by canceling out

synergistic pleiotropic effects, and in doing so reduce genetic correlations, a process known as hidden pleiotropy (Pavlicev and Hansen 2011; Turelli 1985). While hidden pleiotropy can allow traits to be uncorrelated while sharing allelic effects, it can still constrain the evolution of uncorrelated traits. This is possible because the change in allele frequency in pleiotropic loci due to directional selection in one trait can increase the variance in a second trait that is under stabilizing selection, and in doing so increase genetic load, even if the mean of the second trait does not change (Baatz and Wagner 1997). If we consider non-linear GP maps, the situation is even more complex, as the mean pleiotropic effect also has the potential to change the covariance pattern (Mitteroecker 2009).

When considering the effects of the GP map on evolvability, Hansen (2003) points out that evolvability can be attained from genetic architectures other than a modular one, and that independence between traits must be balanced with the allelic effects needed to provide variation. That is, less shared allelic effects between traits leads to the modular variation that allows the traits to evolve independently, but more shared allelic effects increases the amount of available variation for selection. A GP map that maximizes evolvability must be somewhere in between, providing both independence between traits and variability for each trait. If we allow antagonistic pleiotropy, then a highly pleiotropic GP map, with all genes affecting all traits, can produce modular variation and maximally evolvable, because antagonistic and synergistic pleiotropic effects can cancel out and produce a modular covariance pattern that also has a large amount of independent variation in all traits. In summary, antagonistic pleiotropy allows generalized pleiotropic effect to produce modular variation while still contributing to trait variance. Hansen (2003) and Pavlicev and Hansen (2011) explore the conditions under which highly modular or highly pleiotropic GP maps provide higher conditional evolvability, defined as the variation available to respond to directional selection in one trait when the other traits are under stabilizing selection. In a two trait system, the two extreme architectures can both evolve when we consider directional selection on one trait and stabilizing selection on the other. If stabilizing selection is stronger, the modular architecture is favored since the

penalty for correlated response is higher and the modular system provides a smaller unit of selection. If directional selection is stronger than stabilizing selection, the added per trait genetic variance from additional pleiotropic effects can compensate the maladaptive correlated response in the trait under stabilizing selection. Under stabilizing selection on both traits, details of the distribution of allelic effects and the strength of stabilizing selection on each trait can determine which genetic architecture provides higher evolvability (Pavlicev and Hansen 2011). Under the house-of-cards approximation (Turelli 1984) for the distribution of allelic effects, any asymmetry in the stabilizing selection strength between traits can result a modular genetic architecture. Under the Gaussian approximation (Lande 1980), hidden pleiotropy can evolve in most situations. Both of these approximations are valid under different conditions, and in general under high mutation rates and small mutational effects, the hidden pleiotropy GP map provides higher evolvability, while under small mutation rates and large mutational effects, the modular GP map provides higher evolvability. In summary, when considering short term evolution, both modular and fully pleiotropic GP maps can produce maximally evolvable covariation patterns depending on details of the distribution of allelic effects and the pattern of selection. Modular genetic architectures seem to be more common (Wagner et al. 2007), as well as synergistic pleiotropic effects in morphological systems (Kenney-Hunt et al. 2008), but we still don't understand why.

Conditional evolvability measured on standing genetic variation adequately describes the short term ability of complex phenotypes to respond to selection, but longer time scales require us to also consider the influx of mutational variation, and genetic architecture also affects the long term effect of mutations. For example, a modular genetic architecture allows mutational effects to be restricted to functional groups of traits and reduces the cost of deleterious pleiotropic mutations. In regards to the long term influence of genetic architecture on the response to selection, we only expect genetic architecture to constrain long term evolution if it causes mutational correlation (Chebib and Guillaume 2017). Long term evolution also requires us to understand how genetic architecture evolves. Variation on genetic architecture

has been widely established in recent years, with several works showing the role epistasis has in providing variation in pleiotropic and variational patterns (Cheverud et al. 1996; Cheverud et al. 2004; Pavlicev and Cheverud 2015; Pavlicev et al. 2008; Wolf et al. 2006), variation that can then be selected on. Selection on covariation patterns can be direct, caused by correlated stabilizing selection (Bürger and Lande 1994) which causes the genetic and mutational variation to align with pattern of selection (Cheverud 1984). Epistatic interactions between additive loci provide the necessary variation in the pattern of mutational correlations to allow this alignment to evolve in response to correlated stabilizing selection (Jones et al. 2014). Directional selection can also contribute to the evolution of covariation, and there is evidence that traits that are selected together tend to become more correlated, both in simulations (Jones et al. 2004; Melo and Marroig 2015; Pavlicev et al. 2011; Watson et al. 2014) and in natural populations (Assis et al. 2016; Penna et al. 2017; Roff and Fairbairn 2012). The effect of directional selection on the covariance structure can be summarized in the words of Brazilian writer Pedro Nava: "Experience is a headlight that shines backwards", that is, directional selection can bias genetic correlation such that there is an increase in the (relative) evolvability in the directions of past selection. In this sense, we might think of current pattern of constraints as being adaptations to the evolutionary history of the population (Draghi and Wagner 2008), although this hypothesis is controversial (Lynch 2007a,b). There has also been some effort into directly selecting for changes in the covariation structure, and response to direct selection in covariation can be quite dramatic (Delph et al. 2011), but we lack a comprehensive picture for this type of experiment, and we do not know if this type of selective pressure is common in nature.

The evolution of pleiotropic effects also includes second order constraints on the variation in pleiotropy. Development imposes internal selection on the phenotype, in the sense that there is a certain minimum requirement of producing traits that are compatible with each other. This basic evo-devo restriction determines which variants are compatible with forming a complete functioning individual, and can be stable over macro-evolutionary timescales. Mit-

teroecker (2009) argues that variational modularity over longer timescales can only be obtained through localized developmental factors, because over these time-scales the non-linear aspects of development preclude compensatory antagonistic pleiotropy from being stable. There is very little empirical evidence on constraints in the structure of pleiotropic effects, but see the previous chapter and Pavlicev et al. (2016) for an example of development shaping genetic effects.

In this article we use a combination of individual based simulations and an advanced intercross between inbreed mice to explore the pleiotropic base of a change in covariation structure. To create the intercross, we use several inbreed mice lines that were derived from the directional selection experiment by Atchley et al. (1997). In this experiment, an initial positive correlation between growth traits within the initial population was eroded by divergent selection between selected strains. These selected strains were kept as inbreed lines, and using these inbreed lines, we created an advanced intercross line to investigate the genetic basis of the new covariance pattern across lines. We confront the results from this experimental cross with results from simulations. Using an individual based simulation, we explore how pleiotropic patterns evolve under different selection regimes. We relate the genetic effects to the observed covariation in the simulated populations. We expect the pattern of divergent directional selection to influence the pleiotropic basis of the covariation pattern in both simulated and experimental populations.

5.2 Methods

5.2.1 Simulation model

We use an individual-based model with a large number ($m = 200$) of pleiotropic diploid additive loci controlling four quantitative traits in 5000 hermaphroditic individuals. All loci can potentially affect all traits, and so the effects of each locus can be represented by a 4-dimensional vector in trait space (Fig. 5.1). Individual phenotypes are determined by adding all allelic

effects vectors for each individual, resulting in a population of 4 dimensional phenotypes. Phenotypes are then used to attribute a fitness to each individual according to a Gaussian selection surface defined by a multivariate optimum and a selective covariance matrix. Mating pairs are sampled with probability proportional to their fitness and gametes formed by sampling one allele for each locus. Mutation occurs before gamete sampling and is represented by a small random change in the direction and magnitude of the allelic effect vector. These two types of changes, in direction and magnitude of the pleiotropic vector, can occur at different rates (for a discussion on both types of mutations see Draghi and Wagner 2008). Both mutation types occur with a fixed per-generation per-loci mutation probability. We use a mutation probability for the magnitude of the effects of 10^{-4} and a mutation probability in the direction of the effects (the pleiotropy) of 10^{-5} , so the pleiotropic pattern changes more slowly than the magnitude of the effects. Mutations in magnitude are drawn from a normal distribution with mean zero and variance 0.1 and added to the original vector. Mutations in direction are drawn from a multivariate normal distribution with zero mean and a diagonal covariance matrix with variances set to 0.1. In the case of a mutation in direction, after adding the mutation to the original effect vector, the resulting vector is scaled to keep the original magnitude. Because the variance of the added vector is small this produces a small change in the pattern of pleiotropy. All loci are unlinked and inherited independently. Correlated stabilizing selection is applied using a selection covariance matrix with non-zero off-diagonal elements. Directional selection is applied by using a moving optimum. These selective regimes were chosen because all of them have been previously implicated in the evolution of variational modularity in different simulation models (Jones et al. 2014; Melo and Marroig 2015). Both directional selection regimes require some independence between traits in the two modules, as they must change independently in order to respond to the applied selective pressure.

We use three selection regimes to investigate the effects of evolutionary history on the genetic architecture of variational modularity. Populations are first subjected to several generations of drift, then correlated stabilizing selection with two modules. Module M1 composed

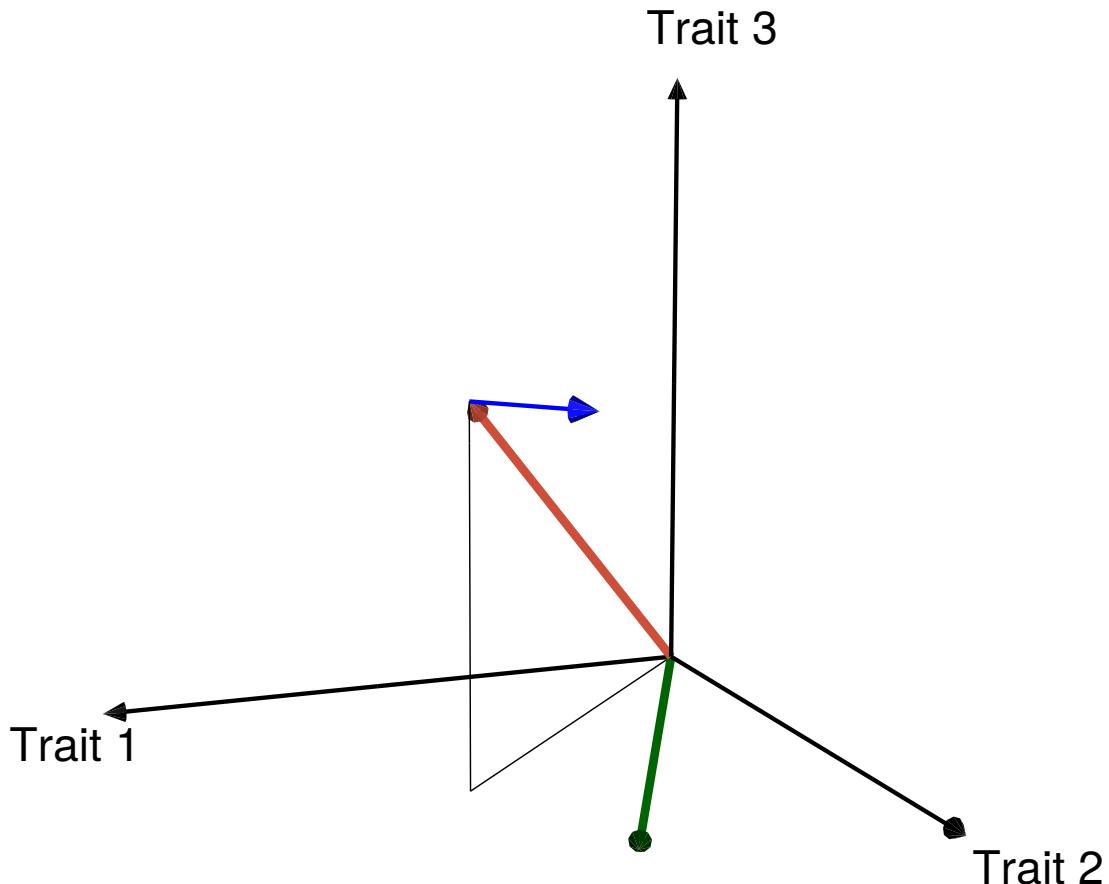


Figure 5.1: Representation of vectors of genetic effects in trait space. Red vector is an integrative vector, while the green vector is modular with respect to traits 1 and 2. Blue vector represents a possible mutation that alters the direction of the red effect.

of traits 1 and 2 and Module M2 of traits 3 and 4. Within module correlation in the selective covariance matrix is set at 0.8, between module is set at zero. After stabilizing selection, in two of the simulated scenarios, we apply directional selection. We use two directional selection modes (1) under divergent directional selection the two modules are selected in opposite directions; (2) under corridor selection, one of the modules is under directional selection while the other is under stabilizing selection (Fig. 5.2).

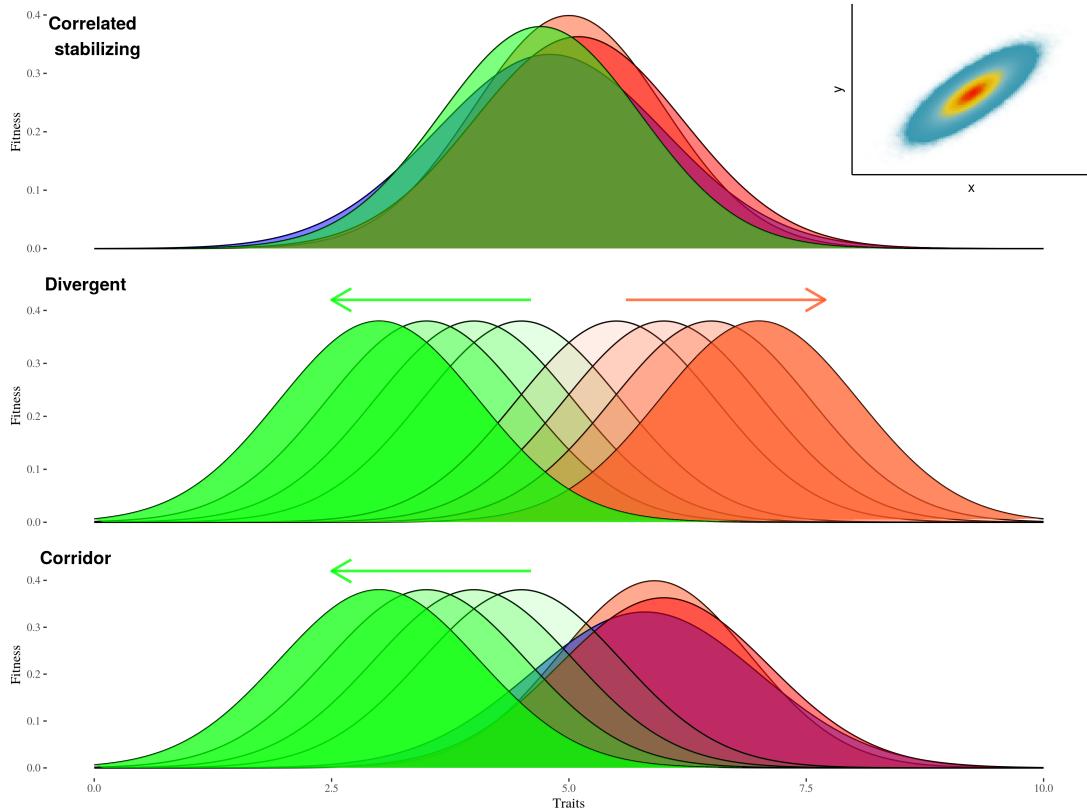


Figure 5.2: Representation of the simulated selection regimes. The Gaussian individual selection surface is defined by two parameters: the optimum and the selective surface covariance matrix. Under correlated stabilizing selection, the multivariate optimum for each trait is kept constant and the selective surface covariance matrix has non-zero off-diagonal elements that produce selection for positive correlations within modules, between traits [1,2] and [3,4] (upper right corner panel). Under divergent directional selection, the same correlated covariance surface is combined with a moving optimum. The optimum for traits [1, 2] is increasing, while the optimum for traits [3, 4] is decreasing. Under corridor selection, we again use the same correlation matrix, and the optimum for traits [1, 2] is increasing, while the optimum for traits [3, 4] is kept stationary.

5.2.2 Mouse lines and phenotypes

Inbred strains

We founded an advanced intercross line using inbred strains derived from a directional selection experiment by Atchley et al. (1997), in which each strain was selected for increase or decrease in their early (0 to 10 days) and late (28 to 56 days) growth rate, with corresponding compensatory selection in the other direction. For example, the E+ selection strain corresponds to an increase in early growth combined with a decrease in late growth. One of our six strains had been selected for fast early growth (E+, A13 strain), two for slow early growth (E-, A22 and A23 strains), one for fast late growth (L+, A31 strain), and two for slow late growth (L-, A41 and A42 strains). Before selection, the original population used in the selection experiment showed an additive genetic correlation between Early and Late growth of about 0.8. After selection, the across selected populations genetic correlation between Early and Late growth was zero. After the selection experiment, the strains were inbred for several generations, and were all <1% heterozygous. Unfortunately, during the several years in which the selected strains were kept in an inbreeding regime, a mistake in the labeling of the animals during a move for a new mice facility led to a contamination of one of the E- strain (A22) with animals from one of the L- (A42) and the other E- strain (A23). This contamination caused an increase in the heterozygosity in the A22 line. Also, across the intercross, some regions of the genome have only five segregating haplotypes instead of the expected six. We discuss this contamination further in the genotyping section.

Breeding scheme

The six inbred strains were crossed non-reciprocally in every pairwise combination, giving 15 combinations in total. For three of the strains, 3 males and 2 females were used in crosses, each paired with a female or male from one of the other strains. For the other three strains, the reverse was true. This design gives an even balance of all six strains on the autosomes,

and close to an even balance of the strains on the Y-chromosome and the mitochondria. From the F₁, two males and two females from each of the 15 litters were used in mating pairs, giving 30 combinations in total. The crossing scheme was designed such that there was no overlap in the strains that had been crossed to produce the parents in the previous generation. That is, each cross resulted in offspring that were a mixture of four of the strains. Furthermore, the two males from a given litter were mated to females that themselves differed in both of the strains that had been combined to produce them. This maximized the number of different strain combinations, and ensured representation of all six strains continued to be equal in the next generation. All crosses were reciprocal in this generation (i.e. if a male from litter 1 was mated to a female from litter 2, then a male from litter 2 was mated to a female from litter 1). A version of this design that balances the contribution of each of the founder strains was continued up to the F₄. However, each F₂ mouse was a mixture of four of the six strains, therefore it was not possible to form pairs between unique strain combinations in this generation. To ensure all F₃ mice included a contribution from all six strains, one male and one female from each of the F₂ litters were used in non- reciprocal mating pairs, again giving 30 (unique) combinations in total, but pairs were chosen such that the parents overlapped in only two of the strains. This resulted in a double dose of two strains in each F₃ offspring. To avoid unequal distribution of the strains in the population, we ensured there was an equal number of each pair of strains overlapping across the 30 pairs. For the F₄ generation, one male and one female from each of the F₃ litters were used in mating pairs, again giving 30 combinations in total. Each F₃ mouse was a combination of all six strains, with a double dose of two strains. Pairs were chosen such that the male and female did not overlap in the strains they had a double dose of (i.e. all F₄ offspring ended up with a deficiency of two strains). To avoid unequal distribution of the strains in the population, we ensured there were equal numbers of litters deficient in each pairwise combination of strains. All crosses were non-reciprocal. One pair failed to produce any offspring, resulting in a very slight imbalance in the representation of the strains. For generation F₅ and F₆, matting pairs were combined at

random, but avoiding within-litter mating and excluding duplicate litter combinations. Two males and two females from each F_4 litter were used in mating pairs, giving 58 combinations (due to one F_3 pair failing to produce a litter). Finally, three males and three females from each F_5 litter were used in mating pairs, giving 174 unique litter combinations. On the day of birth, F_6 litters were trimmed to a maximum of 10 pups (5 males, 5 females or as close to an even sex ratio as was possible given the numbers born). All litters were cross-fostered between mothers within the breeding scheme within 2 days of birth (usually on the day of birth, but occasionally later if a foster mother was not available). Thus, each female acted as a birth mother and a foster mother, and each litter was reared by a different mother to the one that gave birth to it. In total, we use 1513 animals from the F_6 generation.

Phenotypes

All mice were weighed on the day of birth, then at 3 days, 7 days and then weekly until they were 8 weeks old. To define a low dimensional phenotype related to growth, we used the difference in weight over 2 weeks. So our analyses focus on weight gained over each two week interval ('growth') from one week to eight weeks of age. These growth traits were calculated as the difference in body weight between the weeks that define each time interval (e.g., growth from birth to week 2 is defined as $\text{growth}_{0-2} = \text{weight}_2 - \text{weight}_0$). The additive genetic covariance matrix (G-matrix) between growth traits was estimated by an animal model in ASReml-R v3 (Butler et al. 2009) using the phenotypes of the F_5 and F_6 generation and the population pedigree. Sex was included as a fixed effect.

Genotypes

The genomic data was extracted from the spleens of one mouse for each of the inbred strains. Each strain was then run in each lane of a flow cell and sequencing was conducted using paired end Illumina sequencing. This produced approximately 487 million total bases for each sample, all of 125bp in length. Galaxy (Goecks et al. 2010) was used to conduct quan-

tity control using FastQC on the untrimmed sequences. The sequence reads were trimmed in trimmomatic (Bolger et al. 2014) using the following parameters: Cropped first 10 bases from each read, trimmed specified adapter sequences, allowed a maximum of 2 mismatches between the adapter and read sequence, palindrome Clip Threshold of 30, simple Clip Threshold of 10, minimum length of adapter detected of 8 bases, and retaining the reverse read to maintain pairing (`keepBothReads=true`). Following the trimming, we removed leading and trailing bases from reads with quality less than 3 using a sliding window of 4 bases and trimmed if mean quality was less than 15. The trimmed transcripts were then paired and aligned to mouse reference genome (mm10) using Bowtie 2 (Langmead and Salzberg 2012). The ‘Local, sensitive’ alignment algorithm was used, with all other parameters left unchanged. After alignment, SNP calling was carried out using SAMtools (Li et al. 2009) against the mm10 reference genome. The tool ‘Pileup’ was used to identify SNPs, with parameters set to default. From this set of SNPs we set out to design a custom SNP array for genotyping in the intercross generations. Our initial strategy for designing the SNP genotyping array was to select private SNPs for all the strains along the genome. Private SNPs are SNPs that are biallelic among the founder strains, with one strain having one allele and the other five strains sharing the alternate allele. If the density of private alleles is high enough, this provides a trivial way of identifying the strain of origin of a particular stretch of the genome in the intercross line. However, the labeling mistakes in the maintenance of the inbred strains (described in section 5.2.2-Inbred Strains) caused some degree of interbreeding between some of the strains. This made selecting the SNPs for the genotyping array a more challenging task, since in some regions of the genome some of the strains lacked private alleles that would lead to the most informative SNP choice. Given this problem, we developed piecewise approach to select, in each segment of the genome, the most informative set of SNPs available. We divided the full genome into equal sized chunks, and in each chunk ranked the SNPs in terms of their information content: first private alleles (5:1 split of the strains), then SNPs where one allele was shared between two of the strains (4:2 splits) and finally SNPs

where one allele was shared between three of the strains and the other between the other three strains (3:3 splits). We classified the SNPs into these broad groups, and then classified them into categories using the strains involved in the splits, so, we have 6 types of 5:1 SNPs, 15 types of 4:2 splits, and 10 types of 3:3 splits. We then selected at most 4 SNPs for each of these 31 classes of SNPs, totaling at most 50 SNPs per chunk. Selection for the SNPs in the same class was done by selecting the most high quality SNPs (as ranked by SAMtools). This resulted in a set of around 50000 SNPs with maximal information for each chunk that were used by Affymetrix to produce a custom SNP array¹. Using this array, we scored 55338 SNPs, of which 43934 were polymorphic in the intercross. These SNPs were scored in 2299 samples divided among the inbred parental strains, the F₁, F₅ and F₆ generation. Quality control was done using the Affymetrix Axiom Genotyping Solution: Data Analysis Guide². Samples with call rate below 97% were discarded, resulting in 2243 samples. SNP quality control was done using Axiom Analysis Suite³, and after standard quality control filtering we arrived at a final set of 33300 high quality biallelic SNPs distributed along the genome. These SNPs were scored in 1513 individuals from the F₆ generation, 629 from the F₅, 50 from the F₁, and 26 parental individuals from the inbred strains. Phasing and imputation of missing SNP calls was done in Beagle 4.0 (Browning and Browning 2007), using the parent-offspring relations among the individuals to inform the imputation.

5.2.3 QTL mapping

In order to link genetic and phenotypic variation, QTL mapping was performed in the F₆ generation of the intercross using a multivariate mixed model implemented in GEMMA v0.97 (Zhou and Stephens 2014). The inclusion of a random effect is necessary to take the relatedness of

¹Fast parallel code for performing this SNP selection can be found in https://github.com/diogro/EL-snp_selection

²https://tools.thermofisher.com/content/sfs/manuals/axiom_genotyping_solution_analysis_guide.pdf, last viewed in September 20th, 2018

³<https://www.thermofisher.com/us/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/axiom-analysis-suite.html>, last viewed in September 20th, 2018

the individuals in the F_6 into account. We estimate the relatedness matrix using the full set of SNPs in GEMMA with a leave-one-chromosome-out approach (Yang et al. 2014). In this approach, when fitting the model to a given SNP, we exclude the chromosome containing the focal SNP from the relatedness calculation. This method increases power to detect genotype phenotype associations as the signal from the focal SNP is not masked by the effect of the relatedness term. GEMMA estimates an additive effect vector of each SNP on the growth phenotypes, and the significance of each of these fixed effects is assessed using a Wald test. As the SNP are correlated, we use the GEC software provided by Li et al. (2012) to calculate the effective number of markers in each chromosome, and, using a Bonferroni correction, establish a per-chromosome significance threshold corrected for multiple tests. We filter all the significant SNPs at the per-chromosome thresholds, and if two or more significant SNPs in the same chromosome are closer than 20cM we select only the most significant SNP in that interval. We also calculate a genome-wise significance threshold that provides additional confidence to some SNPs, but we do not use this threshold in selecting the markers. We then analyze the additive effects of the identified significant SNPs on the multivariate growth traits.

5.2.4 Genetic effects and covariation

In both the simulations and the QTL mapping we have pleiotropic effect vectors that describe the effect of an allele on a set of traits. The pattern of pleiotropy will largely determine the genetic covariation we observe between traits (see Chapter 4). Alleles that increase two traits simultaneously will contribute to the positive correlation between these traits, while alleles that affect traits in different direction will contribute to the negative correlation between the traits. If two traits don't share genetic effects, the contribution of pleiotropy to the correlation between them is zero, and any genetic correlation we observe will be due to linkage or non-additive genetic effects. Working with a known or putative modular partition of the traits, we can classify these genetic effects on multiple traits by the sign of the effects on each of the traits in relation to the modularity hypothesis. For example, an allele that only increases two traits

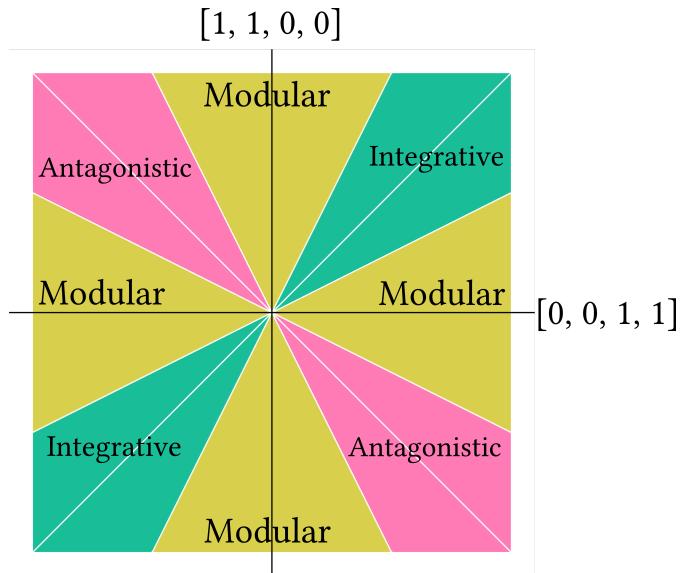
in the same module is defined as modular, while one that increases the traits in one module while decreasing the traits in a different module is antagonistic. We give some examples for four traits in Fig. 5.3, assuming traits [1,2] and [3,4] form modules. When classifying the alleles in the simulations, we first calculate the mean allelic effect at each locus by taking the average of all the alleles at a given loci. Then, we classify these mean pleiotropic vectors in the simulations and the estimated vectors of QTL effects according to their vector alignment with the directions shown in Fig. 5.3, i.e. a vector is classified according to the direction it is most aligned with. In the QTL analysis, we use these effect vectors to estimate the additive genetic covariance matrix directly from all the QTLs using the equations from Chapter 4, and we also estimate the expected covariance matrix from the QTLs classified into a given class.

GENETIC EFFECT CLASSES

Modular	Antagonistic	Local
$[+, +, 0, 0]$ $[0, 0, +, +]$	$[+, +, -, -]$ $[+, +, -, 0]$	$[+, 0, 0, 0]$ $[0, +, 0, 0]$
$[-, -, 0, 0]$ $[0, 0, -, -]$	$[-, 0, +, 0]$ $[+, 0, -, 0]$	$[0, 0, +, 0]$ $[0, 0, 0, +]$

Integrative	Intra-module
$[+, +, +, +]$ $[+, +, +, 0]$	$[+, -, 0, 0]$ $[+, +, -, +]$
$[+, 0, +, 0]$ $[-, -, -, -]$	$[+, -, +, -]$ $[+, 0, +, -]$

(a) Effect classes



(b) Two dimensional classification boundaries

Figure 5.3: a) Pleiotropic genetic effect can be classified according to the direction of the effect on multiple traits with regards to a modularity hypothesis. For example, modular allelic effects can be defined as those that change two or more traits in a module in the same direction, while having no effect in traits outside that module. We also define antagonistic effects as those that have effects in two or more modules in opposite directions, i.e. increasing module 1 while decreasing module 2. Local effects affect only one trait, integrative effects affect two or more modules in the same direction, and finally intra-module effects affect traits in opposite directions inside the proposed modular structure. A non-comprehensive set of examples is shown in panel a) assuming a four trait two module. b) The decision boundaries in the two dimensional plane composed of the two-module directions. Effects along the axis (modules) are modular, effects along the main diagonal are integrative and effects along the secondary diagonal are antagonistic. Intra-modular and local effects do not appear in this plane.

5.3 Results

5.3.1 Simulations

All simulated population responded to the selective regimes. The population under stabilizing selection was kept at the optimum for all traits and showed a two-module covariation pattern compatible with the correlated selection. Both populations under directional selection tracked the optimum closely, changing their mean phenotypes in response to selection (Fig 5.4a). All selection regimes lead to modular covariation matrices, with higher correlations within modules than between modules (Fig.5.4b), however, differences between the selection regimes appear when we examine the pattern of pleiotropy. Stabilizing selection leads to a large proportion of local and intra-module effects, which do not contribute to the observed two module pattern. Modularity under stabilizing selection is due to a combination of a small number of modular effects and a larger number of antagonistic and integrative effects. Under directional selection, there is a large increase in allelic effects aligned with selection, while still maintaining variational modularity. Under divergent selection, most effects are antagonistic, while under corridor selection many effects are modular, but we still see integrated and antagonistic effects. Fig. 5.4c shows the number of effects in each class for all selection regimes.

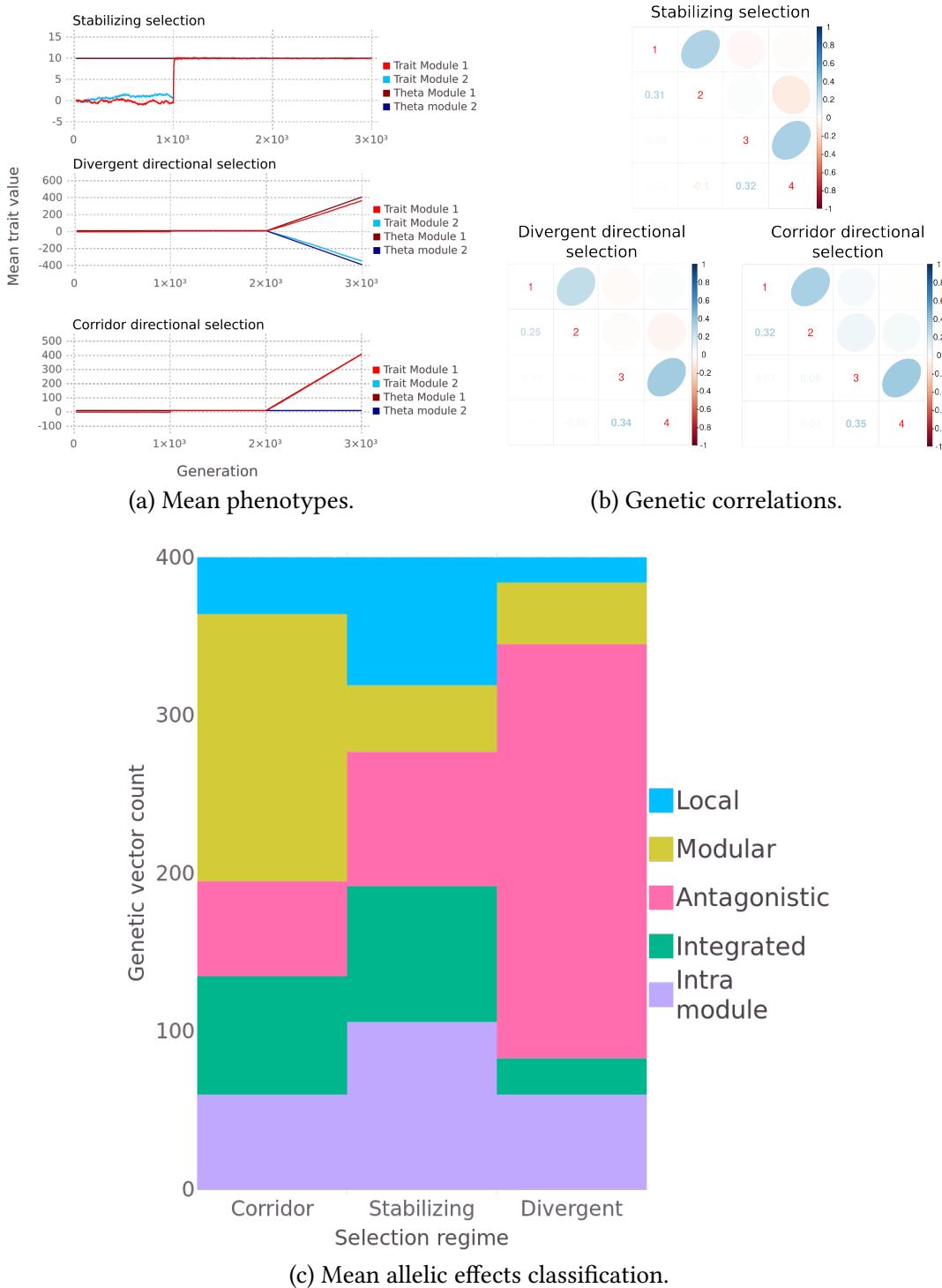
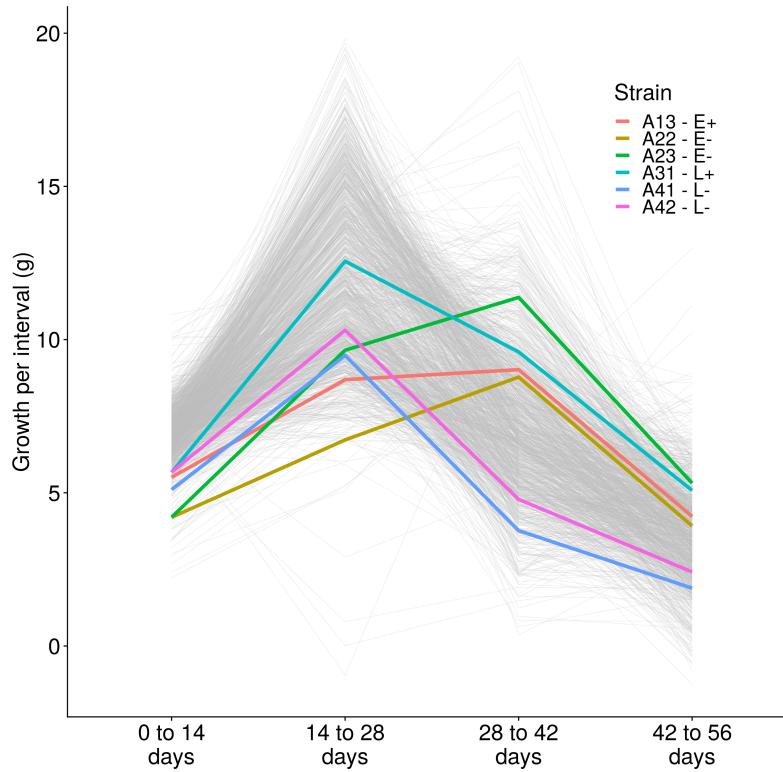


Figure 5.4: Simulation results. a) Changes in the selective surface optimum and corresponding changes in mean phenotypes for the populations under directional selection. All populations start with 1000 generations of drift, then 1000 generations of stabilizing selection with a fixed optimum, then 1000 generations of the corresponding selection regime. b) Genetic correlation matrices for simulated populations. All three matrices for populations under selection show a 2-module covariance pattern. c) Classification of mean allelic effects. Mean allelic effect is calculated over the entire simulated population at each locus, and the direction of the mean effect is classified using vector correlations with the directions in the morphospace.

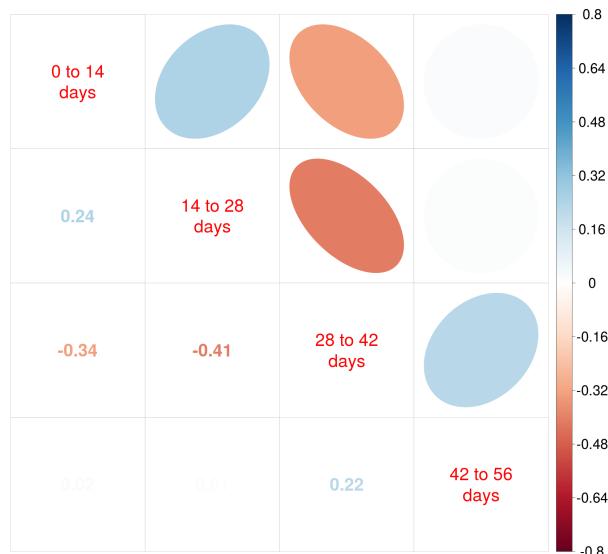
5.3.2 Advanced intercross line

Phenotypes

Mean growth phenotypes measured in the inbreed founder lines and for all individuals in the F₆ are shown in Fig. 5.5a. The F₆ population has more variation in growth than the between line differences, suggesting that interactions and environmental effects are present in the F₆ variation, along with the expected additive variation. One noteworthy difference between the overall distribution of the founders and the F₆ is that, in the founders, three of the six strains have a maximum of growth in the second two week period, while the other three strains have the maximum at the third interval. In contrast, in the F₆ most individuals have a peak in the second interval, suggesting some dominance between strains. Also, in the first two periods, the mean for the F₆ is above the mean for the founders, suggesting that environmental, maternal, or epistatic effects are relevant here. The additive genetic correlation matrix in the F₆ is organized in the familiar two module pattern for early and late growth (as in Chapter 4). We see positive correlations between two traits in each of the two periods, and negative or null correlations between the two phases (Fig. 5.5b). In the ancestral strain used in the selection experiment that produced our founder inbreed strains the correlation between Early and Late growth was positive, so the negative and null correlation we see across the inbreed lines and in the F₆ are a result of changes in the genetic architecture due to divergent directional selection. The negative correlation between the first late interval and the early intervals is strictly speaking not compatible with a modular architecture as there is a dependency between the two phases. In spite of this, we still use this putative modular structure since it is compatible with the pattern of positive correlations and congruent with the artificial selective experiment.



(a) Inbred strains (in color) and F₆ generation (in gray) growth phenotypes.



(b) Additive genetic correlation for growth traits.

Figure 5.5: Growth phenotypes of the inbred founders in color and the full F₆ population in thin gray lines.

QTL effects

We detected 33 pleiotropic QTLs at the per-chromosome 5% significance level, of which 15 were also significant at the 5% genome-wide level (Fig. 5.6, Table 5.1). These QTLs were classified using their directions in trait space according to a putative Early-Late modular hypothesis⁴. All effects vector classes were represented in the detected QTLs: 6 modular, 1 local, 11 intra-module, 7 integrated, 8 antagonistic. We use these effect vectors to estimate the expected covariance matrix from all the QTLs and for the QTLs in each genetic effect class. The full QTL additive matrix, using all detected QTL effects, captures the overall pattern of additive genetic correlations, but the non-null correlations are lower than the ones observed in the G matrix (Fig. 5.5b). Within growth period correlations are positive and larger than the between module correlations. The only difference in pattern is that the correlations between the first interval and the third interval is negative in the G-matrix and positive in the QTL matrix. The other negative correlation, between intervals 2 and 3 is present in both. When we examine the matrix estimated from the QTLs separated into classes we don't see a clear picture. Most of the modular effects are concentrated in the Late traits, and accordingly we see large correlations involving these traits in the modular effects QTL matrix. We do not see a clear modular pattern in the matrix estimated from the modular effects. The intra-module effects produce negative correlations between the third interval and the Early traits. We would expect the integrated effects to produce positive correlations, but we still see two cases of negative correlations. The antagonistic effects also defy our expectation, as they are expected to produce positive within module correlations and negative between module correlations. Instead, we see mostly positive correlations and a single negative correlation between intervals 1 and 3.

⁴We point out that while the Early-Late hypothesis is reasonable and common in growth studies, it is not completely supported by F_6 covariance matrix due to the negative dependency between Early and Late intervals. We continue to use this hypothesis because it is supported by the within-module correlations and is congruent with the selection regimes the inbreed founders are derived from, and so is expected to structure the variation in the F_6 .

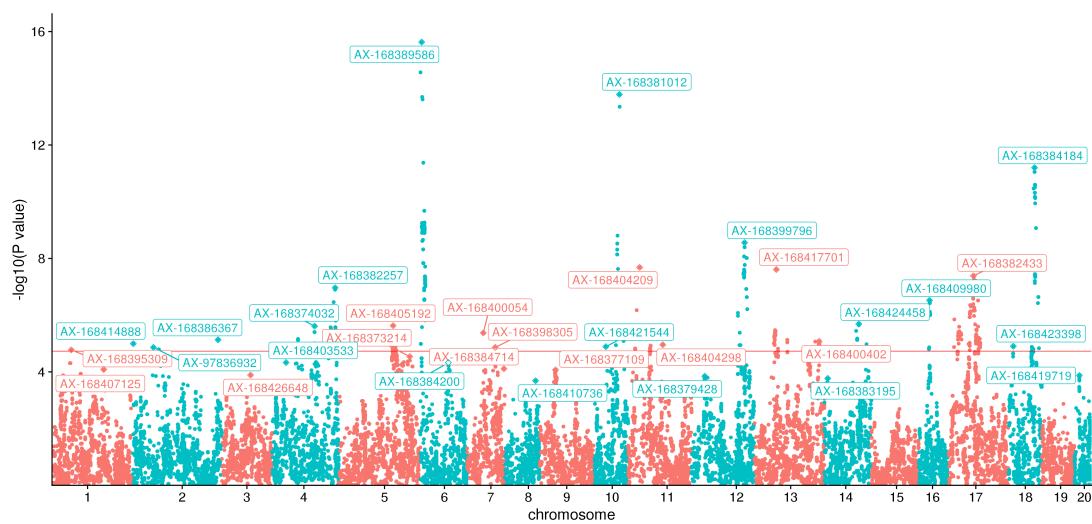


Figure 5.6: Manhattan plot for the p-values in the QTL mapping. Significant SNPs that were selected for analysis are labeled by their ID in the SNP array. The red vertical line marks the corrected 5% genome-wide significance threshold. Alternating colors separate the chromosomes.

Table 5.1: Pleiotropic QTL, their locations in the genome, significance and classification using putative Early-Late modular hypothesis. P-values marked with an * are also significant at the genome-wide level.

	SNP ID	Chromosome	Position (bp)	Position (cM)	Classification	$-\log_{10}(\text{p-value})$
1	AX-168395309	1	49057268	25.84	Modular	4.77
2	AX-168407125	1	125983982	54.05	Intra-module	4.09
3	AX-168414888	2	3732610	2.18	Intra-module	4.99
4	AX-97836932	2	44187081	26.82	Antagonistic	4.86
5	AX-168386367	2	174150478	97.91	Intra-module	5.13*
6	AX-168426648	3	92533605	40.16	Modular	3.89
7	AX-168403533	4	35547379	17.62	Antagonistic	4.33
8	AX-168374032	4	101163884	46.88	Intra-module	5.61*
9	AX-168382257	4	148270976	78.87	Intra-module	6.97*
10	AX-168405192	5	101942137	48.76	Local	5.63*
11	AX-168373214	5	132074919	71.04	Integrated	4.54
12	AX-168389586	6	7605170	3.33	Integrated	15.63*
13	AX-168384200	6	91114085	40.39	Antagonistic	4.33
14	AX-168400054	7	63635159	33.46	Antagonistic	5.37*
15	AX-168398305	7	108326895	54.50	Integrated	4.86
16	AX-168384714	7	140803834	77.20	Intra-module	4.11
17	AX-168410736	8	114057523	58.02	Modular	3.69
18	AX-168377109	9	38023383	20.83	Intra-module	4.07
19	AX-168421544	10	45619439	23.98	Intra-module	4.89
20	AX-168381012	10	97173547	50.38	Modular	13.78*
21	AX-168404209	11	24848821	15.27	Intra-module	7.69*
22	AX-168404298	11	68016486	41.60	Antagonistic	4.96
23	AX-168379428	12	27600863	9.80	Antagonistic	3.85
24	AX-168399796	12	101775963	50.85	Modular	8.56*
25	AX-168417701	13	40086090	19.44	Modular	7.61*
26	AX-168400402	13	111302641	62.25	Integrated	5.08*
27	AX-168383195	14	16548147	7.04	Antagonistic	3.77
28	AX-168424458	14	93468184	45.79	Integrated	5.69*
29	AX-168409980	16	37004375	26.16	Intra-module	6.52*
30	AX-168382433	17	41058707	19.56	Integrated	7.39*
31	AX-168423398	18	15430276	8.63	Intra-module	4.90
32	AX-168384184	18	71316734	45.02	Integrated	11.20*
33	AX-168419719	20	52242051	29.97	Antagonistic	3.87

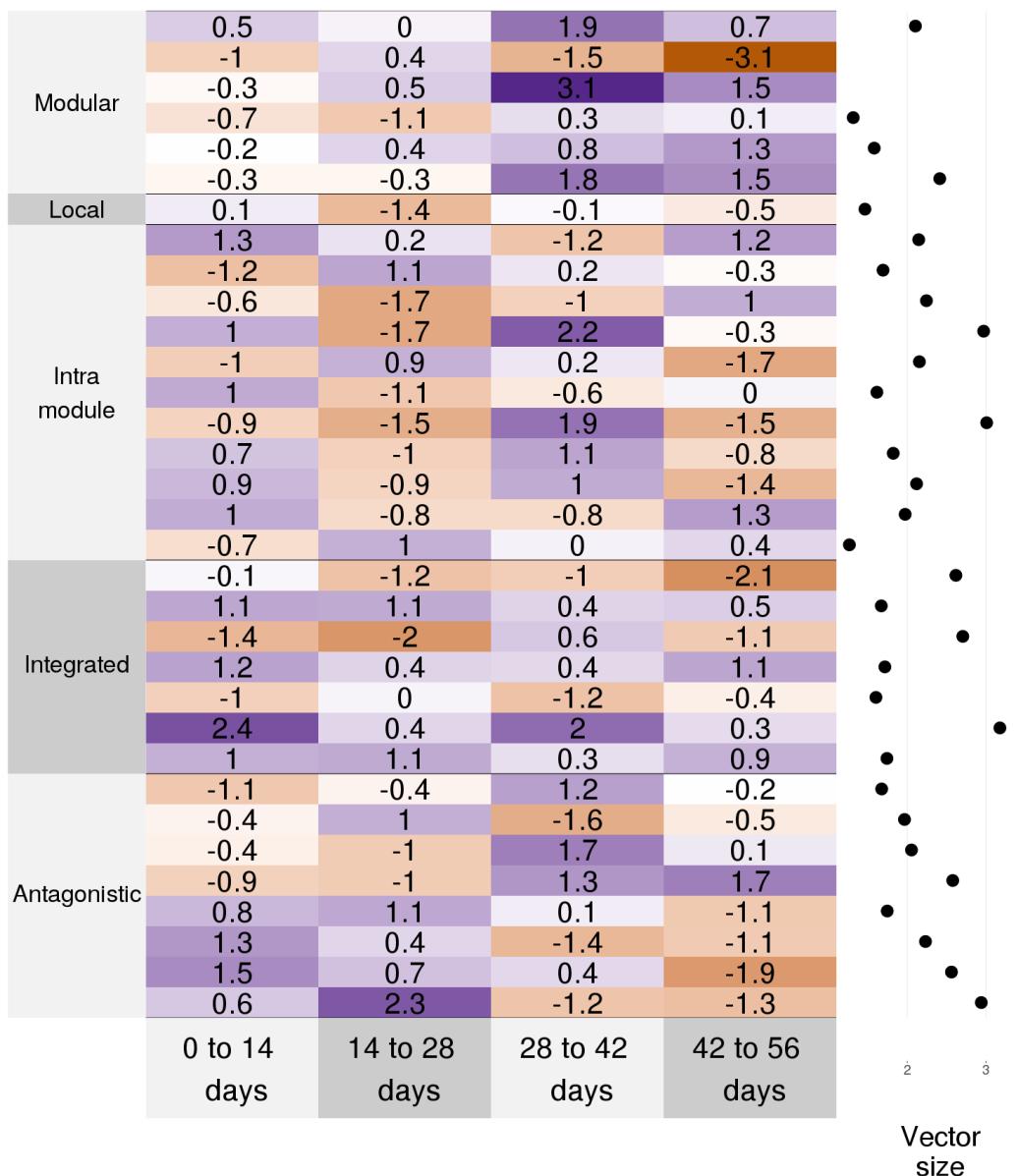


Figure 5.7: Additive effects mapped using multivariate QTL mapping. Each significant marker is associated with a pleiotropic effect vector, and these vectors are classified into the genetic effect classes using alignment with a putative Early-Late modular pattern. Color indicates the sign of the effect, and points show the overall magnitude of the pleiotropic effect. Effects are scaled by trait standard deviation and multiplied by 10.

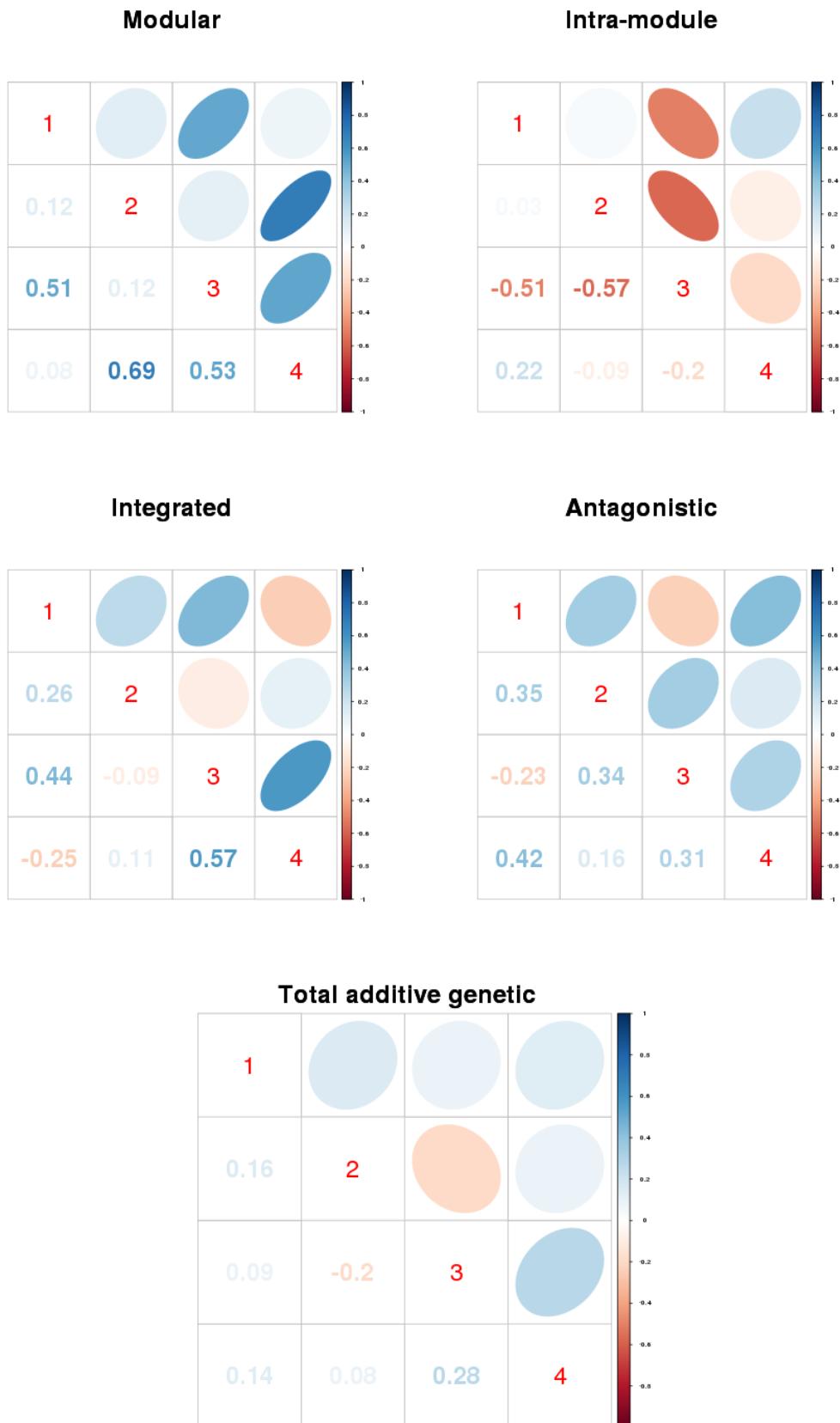


Figure 5.8: Estimated covariance matrices from each class of additive QTL effects, and the estimated matrix from the full set of mapped QTL effects.

5.4 Discussion

In this manuscript we used simulations and an advanced intercross to study the additive contribution of pleiotropy to the establishment of a new genetic covariance pattern. We use simulations and an advanced intercross line and QTL mapping to explore the additive genetic architecture underlying the observed covariance pattern. Even when focusing only on the additive portion of the genetic effects, both simulations and QTL mapping show a complex pattern of modular and antagonistic effects that make up the observed covariation. This underscores the difficulty in uncovering patterns of pleiotropy from genetic covariation alone.

In the simulations, both stabilizing and directional selection are efficient at creating variational modularity. All simulated populations share a similar two-module correlation pattern, but the genetic basis of this modular pattern can change depending on the evolutionary history of the population. In general, a combination of modular, integrative and antagonistic genetic effects interact to produce the final modular variational pattern, but directional selection can increase the proportion of effects aligned with it, altering the GP map. In particular, the populations under directional selection show an enrichment for pleiotropic effects that are in the same direction as the directional selection. Divergent directional selection produces a population with more antagonistic effects, while corridor selection produces an excess of modular effects. These two genetic architectures illustrate some of the possibilities considered in Pavlicev and Hansen (2011), with the population under divergent directional selection presenting the hidden pleiotropy architecture, and the corridor population presenting the modular architecture. The genetic architecture of the population under stabilizing selection appears to be a mixture of these two extremes.

In the advanced intercross line, the F_6 covariance matrix shows a pattern that is markedly different from the original population that underwent divergent directional selection. We observe an erosion of the positive correlation between Early and Late growth as the AIL matrix

shows a distinct pattern of positive correlations within each growth periods and negative or null correlations between periods. This reduction in covariation under directional selection is compatible with what we see in the simulated populations. The negative or null correlations between modules are a reflection of the pattern of directional selection, which selected on an index that altered Early and Late growth in opposite directions. Given the pattern of pleiotropic effects in the simulations, we expected an enrichment of antagonistic pleiotropic effects in the F_6 . This is not what we observe, as the number of modular, antagonistic and integrative pleiotropic effects are about the same (6, 8, and 7, respectively), a pattern more similar to the stabilizing selection regime in the simulations. We also see a large number of pleiotropic effects that do not agree with the modular pattern, the 11 intra-module effects. Collectively, these pleiotropic effects predict a covariance matrix that is broadly similar to the pattern observed in the G-matrix (with the exception of a missing negative correlation between intervals 1 and 3 and the overall lower correlations). In contrast to the good global prediction of the full genetic matrix, the individual effect classes predict covariation patterns that are different from the expected given each class. For example, modular effects predict high between module correlations, integrative effects predict negative correlations, antagonistic effects predict positive between module correlations. Most of the between module negative correlations seem to be due the intra-module effects, which are difficult to interpret in this simple two module scheme. All this suggests that our classification scheme is not accurate enough to separate the different classes of genetic effects in a way that is useful for predicting these different contributions of the genetic covariance matrix. Additionally, the phenotypic distribution of the founder and the F_6 indicate that dominance and epistasis play an important role in shaping growth, as the interval with peak growth differs between these two generations. The heavy use of private alleles in the SNP array and the balanced contribution of the six founders in every individual in the population all but guarantees that most alleles will be far from a frequency of 0.5. This means that dominance effects will contribute to the additive genetic covariance (Falconer et al. 1978; Lynch and Walsh 1998). Since we use only additive

pleiotropic vectors, we are ignoring the contribution of dominance, which could account for the large negative correlations in the G-matrix. Unfortunately, most efficient mixed effect QTL fitting software like GEMMA or FaST-LMM (Lippert et al. 2011) do not include the estimation of dominance effects, but this could be done using custom software, and we plan to include these interaction effects in future contributions.

Attempting to infer patterns of pleiotropy from covariation has been criticized given the many-to-one mapping between pleiotropy and covariation (Mitteroecker 2009; Mitteroecker and Bookstein 2007). Even so, there has been some success in predicting broad patterns of pleiotropy from covariation, for example in Leamy et al. (2002) or Porto et al. (2016). Our results reinforce the difficulty of assessing details of the pleiotropic pattern from genetic covariation, even when we have access to the evolutionary history of the population. We attempted to separate the different contributions of the different direction of pleiotropic effects, but only achieve a reasonable prediction in the aggregate of all the effects. Even though our genetic effect classification scheme is rather coarse and does not allow for a good prediction of the type of correlation pattern within each class, it does reveal the availability of additive variation of different covariance patterns in the intercross population. This variation was established by directional selection and drift in the inbreed lines, which lead to the changes in the original covariance pattern. The covariation pattern in the F₆ is a direct reflection of the pattern of divergent directional selection that produced caused the divergence between the inbreed strains. The resulting modular pattern in the pleiotropic effects of the intercross population is much more complex than the expected pattern given the history of directional selection and our simulation results, even for a simple 2-trait 2-module pattern. We expected a large number of antagonistic effects, that would be aligned with the artificial selection direction, to underlie the modular pattern in the F₆. This is partially not the case, and the genetic covariance pattern is created by a combination of several types of genetic effects in addition to the antagonistic effects, suggesting additional restrictions like development and correlated mutations also play a role in shaping the pleiotropic pattern. However, it is not

clear how much this wealth of variation in covariation due to different types of additive effects would be expected in natural populations. The nature of an AIL combines alleles that have not evolved together and produces within-line variation that was originally between line variation. On top of the combination of additive effects, we also must take into account the many dominance and epistatic interactions that are epiphenomena of the combination of six independent strains. These interaction could be responsible for some of the covariation that is not explained by the additive effects, as we noted above. So, while the GP map in the F₆ might be more complex than in a population that was not an intercross between highly divergent strains, the additive pleiotropic basis of the divergence between strains we observe in the F₆ still gives us insight into how complex the making and breaking of a genetic correlation can be. In the end, simple “modular” or “hidden pleiotropic” patterns are a simplification and that we need to consider complex GP maps to understand the evolution of genetic restrictions.

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Chapter 6

Modularity: Genes, Development, and Evolution

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Abstract

Modularity has emerged as a central concept for evolutionary biology, thereby providing the field with a theory of organismal structure and variation. This theory has reframed long-standing questions and serves as a unified conceptual framework for genetics, developmental biology, and multivariate evolution. Research programs in systems biology and quantitative genetics are bridging the gap between these fields. Although this synthesis is ongoing, some major themes have emerged, and empirical evidence for modularity has become abundant. In this review, we look at modularity from a historical perspective, highlighting its meaning at different levels of biological organization and the different methods that can be used to detect it. We then explore the relationship between quantitative genetic approaches to modularity and developmental genetic studies. We conclude by investigating the dynamic relationship between modularity and the adaptive landscape and how this relationship potentially shapes evolution and can help bridge the gap between micro- and macroevolution.

Keywords: macroevolution, genotype–phenotype map, G-matrix, adaptive landscape, morphological integration

6.1 Introduction

Modularity has become a central concept in evolutionary biology (Wagner et al. 2007). A system is modular if it can be divided into multiple sets of strongly interacting parts that are relatively autonomous with respect to each other. This concept has been applied in developmental biology, in which modules either are different parts of the embryo that interact with each other, as with induction and morphogenesis, or are sets of interacting molecules that act independently in the patterning of multiple tissues. This concept can be extended to adult functional relationships, in which modules consist of parts that act together in the performance of some physiological function. In this review, we focus on the role of variational modules in evolutionary processes. Variational modules are sets of traits that vary together and somewhat independently from other modules.

Modular concepts emerged early in evolutionary thinking with Darwin's consideration of the "correlations of growth" in which he noted that slight evolutionary variations in one part of an organism would result in other parts also being modified. Later, [Weldon (1894), p. 329] noted that "before we can properly estimate the changes at present going on in a race or species we must know ... the degree of abnormality of other organs which accompanies a given abnormality of one". For abnormality read variations. Pearson (1896) then derived the parameter for describing the degree of relationship between two characters that we use today, the Pearson Product Moment correlation.

Despite this very early interest, a multivariate understanding and consideration of evolving characters was not common at the time (Simpson 1958). Certainly before the development of digital computers, the amount of computational work involved in even a small multivariate study on a small sample required the calculation of enormous numbers of variances, covariances, and correlations. These herculean efforts were often not deemed worth the value derived from the research (Simpson 1958). Olson and Miller (1958) stood out by considering the variational relationships between traits as a central feature of evolution and incorporating

a more holistic, systems view of the phenotype and evolution. Olson and Miller (1958) hypothesized that the degree of interdependence in development and function among morphological characters is directly related to their degree of morphological integration as measured by the statistical correlation between trait distributions. Hence, they predicted that developmentally and functionally related traits will be relatively highly intercorrelated. An early example of such phenomena was found in the flower morphology of angiosperms, which can be divided into two sets of highly intercorrelated traits (or correlation pleiades): a vegetative set and a reproductive one (Berg 1960). Further theoretical and empirical work on this concept (Cheverud 1982, 1984; Lande 1979) showed that developmental and functional integration results in correlational selection that leads to genetic integration (genetic correlations). In turn, this genetic correlation leads to evolutionary integration, the correlated evolution of traits. The concept of morphological integration maintained some currency in evolution and systematics from the 1960s through the 1990s. However, interest greatly increased in the new millennium. Much of this increased attention occurred after the publication of several papers on the role of modularity in evolution, especially that of Wagner and Altenberg (1996) and the 1999 University of Chicago Press reissue of Olson and Miller's book, *Morphological Integration*. Wagner and Altenberg (1996) argued that modularity was important in facilitating the evolution of morphological diversity. If all features of an organism are completely integrated, the parts will be prevented from evolving independent adaptations. A modular variational structure permits the evolution of complexity and diversity as observed in the natural world.

Concurrently, important developments were taking place in evolutionary quantitative genetics. In the late 1970s and early 1980s, Lande and colleagues (Lande 1979; Lande and Arnold 1983) reintroduced models of multivariate evolution that had been ignored in evolutionary biology and systematics since Pearson's time (Pearson 1896), although they were better known in agricultural genetics (e.g., Hazel (1943)). Lande (1979) also showed how quantitative genetic evolutionary models could be used in systematics to investigate the evolutionary causes of diversification on a macroevolutionary scale by providing expectations for the diversification

of species under genetic drift and under directional selection. Here we review the genetics of variational modularity, its relationship with development, how it can evolve, and its consequences for evolution and systematics.

6.2 Methodological Considerations

6.2.1 Representations of Morphology

Although most of what is said in this review can be applied to any continuous traits, much of the work related to modularity is concerned with morphological traits. The traditional way of representing morphological structures is to use a suite of linear distances (Olson and Miller 1958), preferably taken within a single homologous structure such as a bone, to represent a given morphological structure in a specimen. This representation captures local developmental and functional factors in a single homologous trait. The last 20 years, however, saw a shift in methodology in favor of using landmark-based methods, especially generalized Procrustes analysis (GPA) (Bookstein 1997; Kendall 1984). GPA takes a set of 2-D or 3-D landmarks measured in a group of specimens, scales all specimens to a common size, and uses an interactive procedure to superimpose the scaled configurations by minimizing the squared distance between the landmarks in all specimens and a mean shape. From this superimposed set we can calculate the distance between each specimen and the mean shape, and this calculated distance is used to represent them. Although this procedure has many desirable mathematical properties (Bookstein 1997) and is a powerful way of describing a morphological structure, its appropriateness for the study of variation and covariation has never been fully established. In particular, because changes in a single landmark will cause changes in the whole configuration, locality of variation is not necessarily preserved in the GPA covariance matrix. This problem has long been recognized in the morphometrics community (Adams et al. 2013), but its consequences for the study of modularity and evolution have only recently become apparent (Márquez et al. 2012; van der Linde and Houle 2009).

Because local variation is not preserved, it is hard to detect local associations and covariation in populations using GPA, thereby limiting its use for the study of modularity. Not preserving local variation is also a problem when relating genetic variation to morphological variation, because again variation will be spread out over the whole morphological structure, and local genetic factors will appear to have widespread effect (Berner et al. 2011). Promising efforts have been made to reconcile landmark-based methods and local variation, such as the local shape variables described by Márquez et al. (2012), finite element scaling analysis (Cheverud and Richtsmeier 1986), and Euclidean distance matrix analysis (Lele and Richtsmeier 1991), but none of these methods have been widely adopted yet. With this problem in mind, we do not discuss approaches that make use of GPA to study covariation and modularity. Instead, we focus on representations of morphology that preserve local variation, like linear distances and local shape variables.

6.2.2 Detecting Variational Modularity

Variational modularity has been used in several different contexts; therefore, a wide range of methods for detecting and quantifying variational modularity in multivariate data are available. At their core, most methods are based on some measure of association among traits (e.g., covariances or correlations), and modularity has often been inferred through the analysis of patterns and magnitudes of association (e.g., Armbruster et al. (2004); Porto et al. (2009)). Given a set of traits in a population and a correlation (or covariance) matrix between them, we might ask which sets of traits are grouped in modules, or if a particular partition of traits is supported by the observed statistical between trait associations. We discuss methods for the detection of modules in these two situations: (a) extracting putative groupings of traits without a prior hypothesis and (b) testing if a particular partition established on different grounds is supported by the observed correlation matrix.

Detecting putative modules is very common in systems biology (Ayroles et al. 2009; Ihmels et al. 2002), in which traits are frequently expression data for thousands of genes and a

priori hypotheses are impractical or impossible. Methods for partitioning traits into modules usually derive from network and graph theory. These methods either treat the correlation matrix as a fully connected weighted graph and use algorithms designed for community detection in graphs (Langfelder and Horvath 2008; Reichardt and Bornholdt 2006) or clustering algorithms coming from other contexts (like Potts model clustering or neighbor joining). Network-based models search for partitions in which members in the same partition share more connections than expected in a random network. Currently, these methods work well in high-dimensional problems, in which misclassification of some individual traits is not a serious problem. Although these methods have been used in much lower-dimensional problems (Magwene 2001), results are not always easy to interpret and partitions can group seemingly unrelated traits together. This can be partially explained by the origin of the methods: Because these methods are borrowed from graph theory, most of the methods and definitions relate to properties of random graphs and how to translate these assumptions to correlation matrices is not obvious. For example, few methods consider the possibility of a trait belonging to two modules, or that modules might have a nested or hierarchical organization. Recently some effort has been made to produce module detection algorithms tailored for correlation matrices (MacMahon and Garlaschelli 2015), but these algorithms have not been applied to biological systems, and more work is needed to develop tools that can deal with complex modularity structures.

In morphological systems with lower dimensionality, and for which information on development or function of the measured traits is available, we may use this information to formulate putative partitions of the traits into modules. These a priori partitions can then be ranked by the support given to them by the observed associations between traits, or tested for compatibility with the observed associations using a significance test. One approach to this problem is to compare the proposed partition with random partitions, using some statistic dependent on the partitions and the correlation matrix. The correlation test proposed by Cheverud (1989) compares the within-module correlations with between-module correlations.

If the observed difference in within- and between-module correlations is higher than the difference for random partitions, the modular structure is considered valid. The RV coefficients (Klingenberg 2009) are a generalization of the squared Pearson correlation coefficient to multiple dimensions and can be used to quantify the degree of independence between two groups of traits. The RV statistic is calculated for a proposed partition and compared with random partitions via permutations. Although this statistic was proposed in the context of landmark data, it can be used with linear distances or local shape variables. Márquez (2008) presents a framework that allows the simultaneous testing of many competing modularity hypotheses, including overlapping and hierarchical modules. The main idea is to use a modularity hypothesis to generate a modeled covariance matrix, in which within-module covariances are set to the observed values and the between-module covariances are set to zero. This modeled matrix is compared with the original covariance matrix with a multivariate measure of similarity. A recent and perhaps more straightforward approach based directly on the correlation matrices and model comparison was published by Goswami and Finarelli (2016).

Both module detection and module validation using correlations are made difficult by the presence of global integrating factors, like size variation or growth, that increase between-module correlations (Mitteroecker and Bookstein 2007; Porto et al. 2013). These are discussed below.

6.3 Genetics of Modularity

Genetic associations among traits can be explained by two different phenomena: pleiotropy and linkage disequilibrium. Linkage disequilibrium (LD) refers to the nonrandom association of alleles at different loci. In large populations and in the absence of selection, LD will be eliminated by recombination after several generations of random mating. For that reason, LD is considered a transient source of genetic association (Cheverud 1996), except in species with only a few segregating chromosomes. Pleiotropy, in our context, refers to the manifold

phenotypic effects of a single unit of inheritance (Stearns 2010). The word context is used here to emphasize the difficulties in finding a universal definition for the term (see Paaby and Rockman (2013)). Pleiotropy, when defined in this way, is considered an important source of genetic association, because it causes traits to be inherited together and, depending on the structure of pleiotropic effects of other contributing loci, to vary together within populations.

Given the importance of pleiotropy as a source of association among traits, one might be interested in the structure of pleiotropic effects of loci underlying modular trait variation (Fig. 6.1) (Wagner and Altenberg 1996). Two prominent questions are whether pleiotropic effects are also modular and whether modular pleiotropy facilitates evolvability, the ability of a population to respond in the direction of selection (*sensu* Hansen (2003)). From a theoretical standpoint, several models for the structure of the genotype–phenotype (GP) map have been put forward (Hansen 2003; Mitteroecker 2009; Pavlicev and Hansen 2011). The general consensus is that, given certain assumptions, multiple different models of the GP map are equally capable of explaining observed genetic associations among traits (Mitteroecker 2009). Similarly, although modular GP maps can maximize evolvability in stochastic environments, they do not necessarily maximize it under more stable environments (e.g., Hansen (2003)).

A clearer picture of whether GP maps are modular and whether they promote the evolvability of organisms came with the collection of large empirical data sets in mice, yeast, and nematodes (see Wang et al. (2010)). These large data sets allowed for a systematic investigation of the pleiotropic effects of genes on the phenotype across a variety of approaches, including quantitative trait loci (QTL) mapping and gene knockout studies. The picture emerging from these large data sets is that most mutational effects are modular, with different sets of genes affecting different sets of functionally and developmentally related traits (Wang et al. 2010). In other words, the variational modularity observed in the phenotype can be explained by modularity in the GP map (*sensu* Wagner and Altenberg (1996)). A minority of mutations affect large groups of traits as they are associated with global genetic factors (Fig. 6.1). More importantly, in these same studies, modular pleiotropy was shown to maximize the rate of

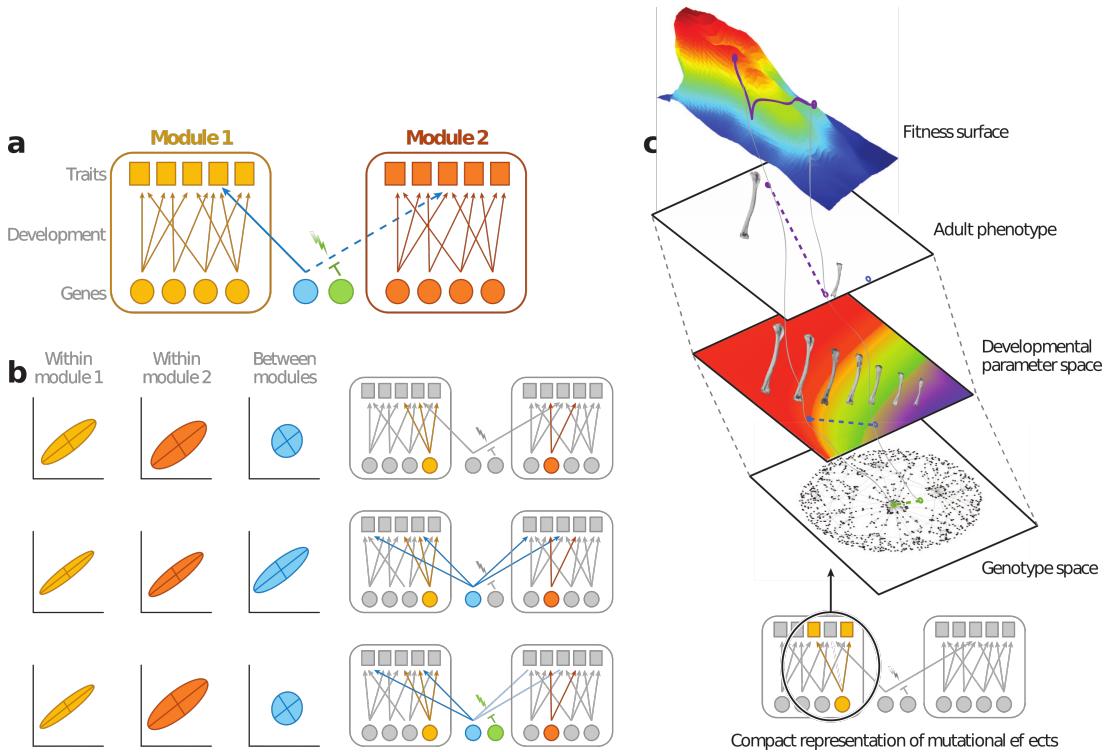


Figure 6.1: (a) Typical representation of modularity in the genotype-phenotype map. Yellow and orange circles represent modular genetic factors, and blue circles represent global ones. The green circle represents a genetic locus capable of preventing the global factor from affecting module 2 (rQTL). Squares represent phenotypic traits, and arrows represent the relationship between genotype and phenotype (polygeny and pleiotropy). (b) Trait correlation as a function of the underlying genetic variation. In case 1 (first row), genetic variation is only present for local factors. Consequently, modular patterns of covariation emerge in the phenotype. In case 2 (second row), genetic variation in both global and local genetic factors is present. Consequently, covariation patterns are less modular. Finally, in the third case, modular covariation patterns emerge again as a consequence of the rQTL preventing the global genetic factor from affecting module 2. (c) The nature of gene effects across the different levels of the biological hierarchy. In this panel, we present a case in which a certain mutation, represented at the level of the genotype, causes changes in the developmental parameter space (e.g., rate of cell division), which in turn leads to changes in the selected phenotype and movement along a fitness surface. Abbreviation: relationship quantitative trait loci (rQTL).

adaptation and promote the evolution of complexity, owing to the scaling of mutational effects with the degree of pleiotropy (Wang et al. 2010).

6.4 Evolution of modularity

6.4.1 Genetic Variation in Pleiotropy

Modularity can evolve through changes in the pleiotropic effects of alleles on traits themselves. Genetic variation in pleiotropy has long been recognized as playing an important role in evolutionary processes. Mayr (1963) noted the importance of epistatic interactions in ameliorating the deleterious pleiotropic effects of alleles on fitness and enhancing their positive fitness effects. Variation in allelic effects at a target locus is produced by differential epistasis, a phenomenon in which epistatic interactions between the target and modifier loci on multiple traits differ in their effects from one trait to the next (Cheverud et al. 1996; Pavlicev et al. 2008, 2011b). Several examples of differential epistasis have been recognized during the last 30 years. The abnormal abdomen (*aa*) locus in *Drosophila mercatorum* is a classic example of this phenomenon. In laboratory experiments, the *aa* locus was found to have a wide variety of pleiotropic effects on morphological and life history traits (Templeton et al. 1985), but these effects were not manifested in wild populations due to modifier loci. Differential epistasis has also been described in several other systems, including coronary artery disease (Maxwell et al. 2013) and viral reproductive success (Pepin et al. 2006).

Although the importance of epistasis has long been appreciated in evolution, only recently has the major part that epistatic pleiotropy plays in shaping covariation become apparent. Wolf et al. (2005) used QTL mapping in experimental crosses of inbred Large (LG/J) and Small (SM/J) mice strains to investigate the genetic architecture in several late and early skull traits. Covariation between traits was strongly affected by epistatic variation in pleiotropy, and the genetic architecture determining the pattern of association between traits can be attributed to a complex pattern of genetic interactions. In these mice, most epistatic effects on pleiotropy reduced covariation and led to a more modular genetic organization. Pavlicev et al.

(2008) investigated the allometric relation between body weight and long bone length in mice, and the authors identified several relationship QTLs (rQTLs), which are QTLs that do not necessarily affect the mean value of traits but affect the relationships between traits (Wagner et al. 2007). This widespread evidence of genetic variation in the covariation between traits due to epistatic interactions provides ample scope for natural selection to change associations between traits and, hence, modularity patterns.

6.4.2 Modeling Changes in Covariation

The availability of variation in the associations between traits led Pavlicev et al. (2011a) to develop a deterministic model for the evolution of pleiotropic gene effects under directional selection. In this model, there is genetic variation in the strength of the correlation between two continuous traits in the form of a polymorphic rQTL that has no effect on the trait mean. Traits that are selected in the same direction tend to become more strongly correlated, even if selection is fluctuating. Conversely, if the two traits are under corridor selection, in which one of the traits is selected to either increase or decrease and the other is kept constant, the rQTL allele representing low correlation is positively selected and the traits become independent. The main conclusion of their model is that the nature of pleiotropic allelic effects is expected to evolve to match adaptive patterns of selection.

Using the existence of variation in pleiotropic relations, Melo and Marroig (2015) developed an explicit individual-based stochastic model for the evolution of continuous traits in finite populations, in which pleiotropic associations between genetic loci and phenotypic traits are free to change under mutation. This model has the advantage of being able to include a number of complications, such as a large number of traits, drift, different patterns of selection, recombination, and mutation. The possibility of simulating several traits is especially interesting because it permits the investigation of complex modular patterns. The authors evaluated the evolution of modularity under a series of evolutionary scenarios and reached a number of conclusions. Drift and stabilizing selection were not capable of creating lasting modular

patterns of covariation, whereas divergent directional selection (during which one group of traits is selected in one direction and another group is selected in the opposite direction) created modularity—traits selected in the same direction became more strongly correlated and formed clear variational modules. Under corridor selection, the group of traits under directional selection becomes more correlated, whereas the group of traits under stabilizing selection maintains intermediate levels of correlation, and the correlations between these two groups become very low. These findings suggests corridor selection is a powerful mechanism for creating complex modular patterns.

Epistasis has also been implicated in the evolution of the mutation matrix for continuous traits. In Jones et al. (2014) the authors developed a model for the evolution of two continuous traits under genetic control of several pleiotropic loci. The model also included a stable pattern of epistatic interaction between the loci affecting the quantitative traits. The traits were subjected to correlated and independent stabilizing selection. Under uncorrelated selection the traits presented correlations near zero, and the mutation matrix also had zero correlations between traits. Under correlated stabilizing selection, however, the traits' genetic correlations changed and mirrored the pattern of stabilizing selection. The mutation matrix also aligned with the selection surface and led to a situation in which the effects of new mutations are biased by previous selective history.

These different models allow us to draw general conclusions regarding the expected evolution of covariation between quantitative traits, regardless of the specific model used. First, variation in pleiotropic relations is essential to the evolution of modularity, and this variation can be attained through epistatic interactions. Second, under directional selection, traits that are jointly selected in the same direction tend to become more strongly correlated. Third, because selection can change the pleiotropic and developmental relations between traits, future evolutionary changes can be biased by previous selective history.

6.4.3 Empirical Evidence for Changes in Covariation

Several instances in which evolution, and presumably selection, has broken down patterns of association among traits to produce major adaptive shifts have been reported. Hallgrímsson et al. (2012) classified these changes in patterns of modularity as a form of evolutionary novelty, as there is “a breakdown of ancestral developmental constraints such that variation is generated in a new direction or dimension.” Indeed, this type of novel variation has been documented in several systems. Young and Hallgrímsson (2005) found a common pattern of strong covariation between the forelimb and hindlimb elements in quadrupedal mammals that constrains the independent evolution of the limbs. However, two mammals with highly derived limb morphologies, the brachiating gibbons and the flying bats, show a reduction in the cross-limb correlation that accompanies their extreme limb individuation.

Examples of artificial selection overcoming the initial pattern of genetic associations have also been found. Beldade et al. (2002) used the eyespots in butterfly wings as a target of selection. Initially, the anterior and posterior eyespots were correlated, and selection for coordinated change of both eyespots produced a rapid and linear response; whereas selection in the uncoupling direction, for increase in one eyespot and decrease of the other, lead to a response that was much less linear and more irregular. This study illustrates that there are preferential directions for evolution produced by the pattern of modularity, and that evolution is faster in these directions, but these are not absolute restrictions. Similar results were obtained for artificial selection experiments aimed at reducing between-sex genetic correlations for flower size in *Silene latifolia* (Delph et al. 2011). Recently, artificial selection experiments using *Drosophila melanogaster* revealed yet another important role for selection in influencing patterns of association among traits. By selecting on allometric relationships in drosophilid wing shape, Bolstad et al. (2015) produced laboratory lineages presenting larger differences in allometric slopes than the ones observed across a large clade of drosophilids. This evolutionary response to selection in the allometric slopes was, however, quickly lost after selection pressures were suspended. This finding indicates that internal selection might

be responsible for maintaining conserved allometric slopes on a macroevolutionary timescale.

These results illustrate the complex interactions between modularity and selection. Evolutionary restrictions imposed by genetic associations are rarely absolute, and selection that privileges uncoupling of associated traits can lead to a reorganization of variational patterns. At the same time, covariation patterns can also be largely maintained due to either internal selective pressures (as in the allometric relations in *drosophila*) or differences in the availability of rQTL variation to change pleiotropy.

6.5 Development as the Link Between Genes and Phenotype

Understanding the mechanics and regulation of development is becoming increasingly essential to elucidating the relationship between modularity and trait evolution (e.g., Salazar-Ciudad and Jernvall (2010)). Development occurs not only through the molecular interaction among many gene products in a particular environmental context but also through the mechanical interactions between the developing cells and tissues, all of which can create significant non-linearities in the GP map (Alberch 1991; Polly 2008; Watson et al. 2014). In this section, we review recent literature that explicitly addresses the connection between quantitative approaches to modularity and the underlying developmental genetics. We are particularly interested in studies that explicitly incorporate the mechanics of development into an evolutionary framework or that identify genes that contribute to change in canalization. We also highlight the emergence of the new field of system genetics.

6.5.1 Causes of a Phenotype Versus Causes of Phenotypic Variation

In a developmental context, it is particularly important to distinguish between the causes of a phenotype and causes of phenotypic variation. A developmental process might be essential for a trait to emerge (cause of a phenotype), but as long as this process is conserved across individuals, it will not be a cause of phenotypic variation in a population. Empirical evidence

overwhelmingly suggests that causes of a phenotype are modular in nature. The whole concept of character relies on it. According to Wagner (2007), character identity is specified by gene regulatory units that control the developmental program. These regulatory units, termed character identity networks, imply that we can truly recognize something as a distinct character only if it shows some degree of modularity at the developmental genetic level.

Modularity in the causes of variation of a phenotype is another matter altogether. The notion that causes of phenotypic variation might also be modular stems from the concept of morphological integration, as seen above (section *Genetics of modularity*), and from the imitative epigenotype hypothesis (Riedl 1978), which predicts that the pattern of developmental constraints imitates the pattern of functional constraints, thereby leading to covariation among functionally related traits within populations. Empirical evidence suggests that there can be a correspondence between variational and developmental modularity, but this correspondence is by no means guaranteed, as seen in previous sections.

6.5.2 Incorporating Development into Evolutionary Studies

Although variational modularity is often assumed to be a consequence of variation in the underlying developmental mechanisms, explicitly modeling developmental systems or even inferring developmental processes from variational modules are not simple tasks (Hallgrímsson et al. 2009; Pavlicev and Cheverud 2015). Interactions among tissues, as well as local and global genetic factors acting at different time points, are all superimposed during development and contribute to the final phenotype (Hallgrímsson et al. 2009; Mitteroecker and Bookstein 2007). Similarly, a single modular pattern can emerge through multiple independent developmental pathways (Mitteroecker 2009) and make the prediction across levels of the hierarchy difficult. To our knowledge, the most successful empirical case of incorporating developmental parameters in evolutionary models is Salazar-Ciudad and Jernvall (2002, 2010) model of tooth development. Tooth development is a relatively well understood process, as several of the genetic interactions and cellular processes that lead to tooth formation are known. Con-

sequently, Salazar-Ciudad and Jernvall (2010) created mathematical models describing tooth morphology as a consequence of perturbations in the underlying genetic and developmental parameters, such as the rate of cell proliferation or cell adhesion. This model was successful at producing accurate predictions of tooth morphology for several mammalian groups (Salazar-Ciudad and Jernvall 2010). Other successful cases of the use of developmental mechanisms to explain phenotypic variation comes from *Drosophila* wing venation patterns (Matamoro-Vidal et al. 2015) and butterfly wing eyespots (Beldade et al. 2002), both of which involve changes in several key developmental processes, such as the distribution of morphogens in the wing disc or the establishment of planar cell polarity.

A theoretical approach that has also undertaken a more explicit incorporation of developmental information into the evolution of the GP map was developed by Watson et al. (2014). In their model, multivariate traits are produced by a GP map with multiple independent developmental steps connecting the phenotype to the genotype. This conceptualization produces a nonlinear ontogeny and allows the model to capture interesting behaviors of these GP maps, such as the ability to recall multiple phenotypes that were selected in the past or the ability to produce new combinations of features from modular developmental processes. Traits in this model tend to become more associated throughout development when they are selected in the same direction and become independent when they are selected in different directions, thereby reinforcing the role of directional selection in shaping modularity.

In conclusion, although approaches relating variational modularity to the mechanics of development are relatively rare, these different empirical studies and theoretical models clearly show that incorporating the more complex developmental interactions into studies of morphological variation and evolution greatly increases our ability to understand and even predict the evolutionary dynamics of complex systems. The main challenge going forward will be to create models capable of describing more complex structures, such as the skull; allowing specific connections between DNA sequences, developmental networks, and variational modularity; and incorporating the possibility of changes in the topology of genetic and developmental networks.

6.5.3 System Genetics—A Systematic Approach

A promising way to integrate modularity with the underlying developmental genetics in a systematic way is currently gaining traction with the system genetics approach (Ayroles et al. 2009; Mackay et al. 2009). The idea behind system genetics is simple. It interrogates the relationship between genome and phenotype under different contexts (e.g., environments or conditions). Consequently, it attempts to hit at the core of the context dependency of gene effects, which not only is fundamental for the evolution of modularity (Pavlicev and Cheverud 2015), as seen in previous sections, but also emphasizes its developmental basis by potentially uncovering important modular signaling cascades.

System genetic approaches have been applied to several different model organisms (Ihmels et al. 2002; Juenger et al. 2005; Wang et al. 2010). In *Drosophila* Ayroles et al. (2009), it led to the identification of several transcriptional modules that are not only connected to genomic variation but also underlie variation in ecologically relevant traits, such as fecundity and metabolism. Those transcriptional modules are strongly influenced by environmental, developmental, and genetic background effects, thereby highlighting the fact that context-dependent effects are the norm and therefore are responsible for most phenotypic variation. System genetic approaches have also recently been used to map changes in the amount of variation for a given phenotype (Ayroles et al. 2015). Genetic variation in phenotypic variance represents genetic variation in developmental canalization, a topic that is especially relevant to studies of threshold characters or threshold selection (Ayroles et al. 2015). Among the challenges faced by system genetics, two should be highlighted. Due to its ambitious nature of scoring multivariate traits and entire transcriptomes/genomes, system genetics studies are inherently expensive studies. Also, multivariate statistics are often dependent on large samples and are often estimated with considerable error, an aspect that needs to be taken into account.

6.6 Modularity and the Adaptive Landscape

How is modularity and integration relevant for phenotypic evolution? Having discussed and characterized modularity and the possibility of its evolution, we now address its evolutionary consequences. There are short-term and potentially long-term consequences of modularity for evolutionary change. We start by introducing the quantitative theory dealing with the short-term consequences and identifying under which circumstances this theory can be extended to macroevolutionary time. Because modularity in patterns of genetic associations between continuous traits is captured by the additive genetic variance/covariance matrix, the G-matrix (Lande 1979), this section focuses on the relationship between the G-matrix and the adaptive landscape, which relates possible phenotypes in the morphospace with fitness (*sensu* Arnold et al. (2001); Simpson (1944)).

6.6.1 Why So Much Interest in the G-Matrix?

Evolution, regardless of which evolutionary process is involved, depends on genetic variation. The G-matrix summarizes the amount and pattern of additive genetic variation and covariation among traits and is, therefore, essential to our understanding of the connection between genetics and evolution (Lande 1979). Genetic covariation among traits is particularly important because of its potential to affect the course of phenotypic evolution (Fig. 6.2). Unlike the univariate view of evolution, in which a single trait's value can be optimized without constraint from selection on other traits, genetic covariation among traits causes correlated responses to selection. In this situation traits will change and evolve together, often in a direction that is different from the one favored by selection (Fig. 6.2) (Grant and Grant 1995; Lande 1979). Thus, the pattern and magnitude of the G-matrix elements can deflect the path of evolution from its optimal trajectory. Whether or not this short-term effect on the evolutionary responses has enduring consequences depends on the degree of stability of the G-matrix and on its relationship with the adaptive landscape [Steppan et al. (2002); see also section *Evolutionary change in rugged landscapes*].

Long-term stability of the G-matrix is one of the most fundamental assumptions of the research program described here, and questions related to the long-term stability and estimation of G-matrices are still open (Houle and Meyer 2015; Jones et al. 2012). Many feel uncomfortable with this assumption of stability (Björklund et al. 2013). This discomfort stems in part from empirical evidence that suggests that no two populations have identical G-matrices and that G-matrices can fluctuate over short time periods (Björklund et al. 2013; Eroukhmanoff and Svensson 2011). Biological populations are finite and almost surely differ in their gene frequencies, especially considering the potentially large number of genes affecting complex traits and the correlations between them (Phillips et al. 2001; Whitlock et al. 2002). We suggest that in many instances the assumption that population covariance matrices are identical be rejected out of hand. But the mere presence of a statistically significant difference is not the critical issue. Instead, the more interesting and relevant questions are: How similar are two covariance patterns with respect to their predicted evolutionary responses? Do some quantitative traits have more stable G-matrices than others? Fortunately, these are questions that can be examined empirically (Calsbeek and Goodnight 2009; Cheverud and Marroig 2007) and are a critical first step for the use of the G-matrix in multivariate evolution and systematics.

So, what do empirical studies tell us about the relative stability of the G-matrix over macroevolutionary timescales? Empirical evidence varies greatly depending on the study system in question. One of the most thoroughly explored cases is the mammalian skull, from which empirical evidence strongly suggests that G-matrices are stable across mammalian taxa (Garcia et al. 2014; Marroig and Cheverud 2001; Porto et al. 2009). By stable we do not mean that heritable variation patterns are identical across species, but that they will deflect the phenotypic response to selection in a similar way.

Although it is possible that extant species variation patterns are fairly similar, it is also possible that stochastic fluctuations in the G-matrix over generations are large enough to render the inferences we might make from extant patterns useless. These fluctuations are possible for many reasons, like segregating alleles with large effects or linkage disequilibrium

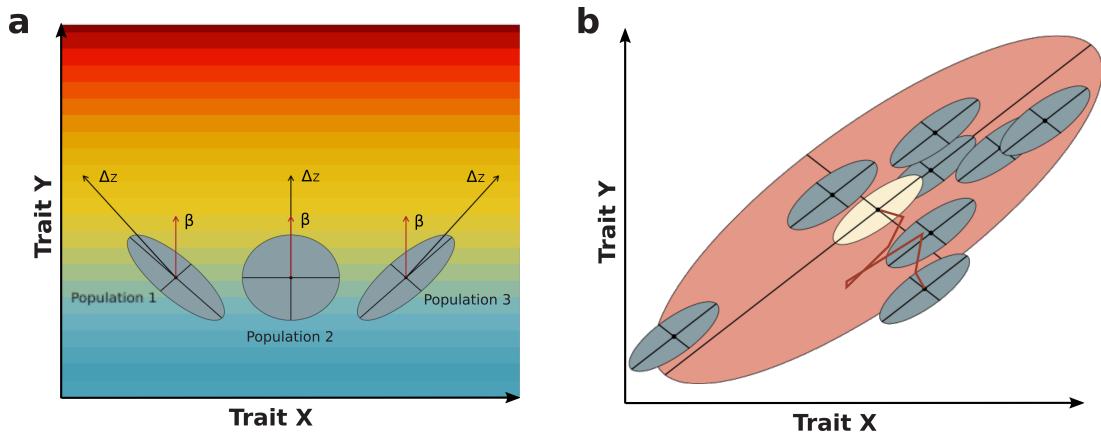


Figure 6.2: To illustrate the interaction of modularity (captured in the G-matrix) and evolutionary processes (selection and drift), we display two panels illustrating population averages, G-matrices, adaptive peak(s), and selection gradients (β). G-matrices are represented by ellipses of different colors and with the axes of major genetic variation embedded. The major axis corresponds to the line of least resistance (Schluter 1996), that is, the direction which holds most of the genetic variation in the trait space. Selection gradients are represented by straight arrows and measure the relationship between fitness and individual traits while holding the other traits constant. Responses to selection (Δz) are also shown as arrows and indicate changes in the trait averages across time. The multivariate response to selection equation ($\Delta z = G\beta$) captures the relationship between the response to selection (Δz), inheritance (G-matrix), and selection (β). The direction of increase in average fitness is indicated by the plus sign (+) and decrease in fitness is indicated by the minus sign (-). The left panel shows 3 populations (green, yellow, and red) under the same adaptive landscape, in which an increase in trait Y is favored and trait X does not affect fitness. Notice that the response of each population to the same selection gradient differs (dark green, orange, and dark red arrows). Population Green increases Y but decreases X values, population Red increases Y and X values, and population Yellow only increases Y, although with a smaller displacement of Y than the other two populations. These different responses under the same selection gradient are due to differences in the G-matrices. Populations Green and Red have their responses deflected from the optimal path, due to the covariation between Y and X (negative in Green, and positive in Red). Thus, traits that are not under direct selection will contribute to the response owing to their shared inheritance. If traits are independent (population Yellow), each can be optimized separately. But note that the displacement in the Y average is smaller than in the other populations, because the genetic variation available in that direction is smaller. The right panel shows the consequence of a flat adaptive landscape (random genetic drift) on the averages of descendant populations (red ellipses) of an ancestral population (yellow ellipse). The arrows in this case point to each population's trajectory. At the end of the drift process, 95% of all evolution (divergence among means) is captured by the larger ellipse (light pink). Notice that substantially more divergence appears along the axis holding most of the within-population genetic variation.

caused by periods of strong fluctuating selection (Bulmer 1971; Turelli 1988). Although this is certainly a theoretical possibility, the critical question is whether or not changes in G-matrix structure are of sufficient magnitude to affect evolutionary inferences (Arnold et al. 2008). Fortunately, simulations quantifying these problems suggest that their effect may often be small (Jones et al. 2004, 2012).

6.6.2 The Missing Link: Adaptive Landscapes and the G-Matrix

We have discussed genetic associations, the genetic and developmental origins of modularity, and their influence in evolutionary response. One missing, and arguably the most important link, is the relation between covariation and the adaptive landscape. By examining the relationship between modularity and adaptive landscapes, we can put micro- and macroevolution into a common unifying theory (Arnold et al. 2001; Arnold 2014). This theory not only explains the relationship between development, function, and inheritance in shaping modularity patterns but also allows us to explore the evolution of multivariate phenotypes in deep-time and suggest future research directions.

Although many studies have been conducted at a local scale (within-population), we have precious little information about how adaptive landscapes vary between species (Pfaender et al. 2016). One of the most important developments in the past 30 years of evolutionary theory were the multivariate regression methods for inferring individual selection surfaces from multivariate data (Lande and Arnold 1983). Although not without critics (Shaw and Geyer 2010), these methods have allowed researchers to directly investigate adaptive landscapes and to empirically measure selection on multivariate trait sets. In this approach, the adaptive landscape is described by two main terms: a linear term related to directional selection and a quadratic term related to stabilizing/disruptive selection (Lande and Arnold 1983). The quadratic component affects the variances and covariances among traits and, along with directional selection, is thought to be the major evolutionary process shaping modularity (Melo and Marroig 2015). The selection gradient (linear term) is the direction of maximum

increase in fitness and is the vector of partial regression coefficients of fitness on traits. This framework can also be used to study directional selection retrospectively: By measuring extant species means and covariance matrices we can estimate ancestral states and, by solving the Lande equation, the selection gradient that would have resulted in the observed diversification can be estimated (Lande (1979), equation 9) (Fig. 6.2).

Even though this toolkit for explicitly characterizing selection has now been available for decades, empirical characterizations of multivariate selection are still rare, despite their acknowledged importance. Yet the past 20 years of reconstructing selection gradients from extant diversity have given us two fundamental insights into the nature of multivariate evolution. First, estimates of the strength of both stabilizing and directional selection are usually weaker than we previously assumed (Kingsolver et al. 2001; Kingsolver et al. 2012). But, more importantly, the empirical evidence suggests that the direction of evolutionary divergence and the direction of selection are rarely the same and, often times, present little resemblance (see the section, Does Alignment with Lines of Least Resistance Imply Constraint?). An important work illustrating this notion comes from studies of *Drosophila serrata* (Chenoweth et al. 2010). In a study of sexual selection, the authors note that even though local processes of sexual selection varied considerably across their nine populations, evolutionary divergence occurred primarily along a single trait combination. Variation in sexual selection had little influence on evolutionary divergence. Instead, genetic covariation among traits caused the evolutionary response to be significantly deflected from its optimal path. Studies on *Dalechampia* blossom morphology have also emphasized that only a portion of evolutionary divergence patterns can be accounted for by estimates of external selective factors, such as community composition and availability of resources. Rather, constraints imposed by covariation patterns seem to be essential for our understanding of the evolution of blossom traits (Bolstad et al. 2014; Hansen et al. 2006).

We should point out that selection gradients reported in these retrospective works are net gradients, that is, estimates of the cumulative sum of all selection gradients acting over the

generations of divergence. If the G-matrix is stable, this net selection should be a reasonably accurate estimate of the sum of individual gradients (Jones et al. 2004). Another issue with reconstructing selection is that G- and P-matrices are often estimated with substantial error, frequently resulting in poorly conditioned or negative semidefinite matrices. Thus, the inversion step in the reconstruction analysis can lead to very large errors (Marroig et al. 2012). Fortunately, matrix estimation or regularization methods can vastly improve the selection gradient estimates, and these methods should be used whenever selection estimates are made (Marroig et al. 2012; Schäfer and Strimmer 2005).

6.6.3 Evolutionary Change in Simple Landscapes

Most of our knowledge of the relationship between modularity and the adaptive landscape comes from simulation studies. In simulations carried out on simple landscapes, patterns and magnitudes of association among traits affect the direction, magnitude, and rate of evolutionary change under selection (e.g., Marroig and Cheverud (2010)). The effect of the G-matrix on evolutionary change depends critically on its structure in relation to the adaptive landscape (Conner 2012; Laughlin and Messier 2015) and can either augment or slow the evolutionary response relative to a situation with fully independent traits. If selection is along dimensions unaligned with modularity/integration patterns, the response is deflected toward the lines of least resistance (Schluter 1996). If selection is aligned with modularity, however, the evolutionary response is greatly facilitated (Beldade et al. 2002; Bolstad et al. 2014). The closer the alignment with the major line of least resistance, the quicker and more direct the evolutionary response. However, simulations are highly concordant in showing that these effects are restricted to the microevolutionary scale, and, given sufficient time and a simple adaptive landscape, the population will eventually reach the selective peak, unless there is no genetic variation at all in that direction (an absolute constraint) (Blows and Hoffmann 2005). But theoretical work suggests that even if there is an apparent lack of genetic variation along some dimension, genetic variation is frequently hidden in the form of epistasis that can fuel

evolutionary change in subsequent generations (Hansen 2013; Hansen et al. 2006). Therefore, given the possibility of adaptive changes in the G-matrix through time and the understanding that constraints imposed by G-matrices are usually microevolutionary, the emerging picture would be one in which G-matrices should not have any enduring macroevolutionary consequences (termed the transient constraints model, from now on). But what happens when we consider complex adaptive landscapes?

6.6.4 Evolutionary Change in Rugged Landscapes

Although single-peaked adaptive landscapes are convenient for model building purposes, adaptive landscapes are thought to be very rugged, that is, they have many adaptive peaks and valleys (Kauffman and Levin 1987; Martin and Wainwright 2013; Pfaender et al. 2016; Wright 1932). When the adaptive landscape is rugged and genetic associations are stable through time, macroevolutionary dynamics are shaped by the interaction between the G-matrix and the adaptive landscape (Fig. 6.3). This interaction implies that, in rugged and multiple-peaked adaptive landscapes, the G-matrix can have a major influence in determining which peak will be reached by a given population, even if in theory the effect of the G-matrix is microevolutionary (Steppan et al. 2002). This argument was already present in Lande (1979, p. 407) but in a somewhat opaque formulation: “[However,] the adaptive topography for each population or species generally has multiple peaks.... Genetic correlations can alter the long-term result of selection by influencing the direction of evolution at critical periods when a population approaches a threshold (or saddlepoint) between adaptive zones, as by random genetic drift or by environmental fluctuations which directly affect the phenotype or alter the adaptive topography.” This argument can be easily understood noting that, in evolutionary terms, the distance between the population average position and the peak is not a simple linear (Euclidean) distance between the start position and end position of the species averages but is a weighted distance, with the weight being given by the patterns of genetic association. Given the influence of genetic correlations, the distance of a population from a

peak is measured in units of genetic variation. Thus, the closest peak, the peak the population eventually reaches, is not necessarily the highest or even the closest in Euclidean distance but is the closest in genetic-scaled distance. We refer to this idea as the peak selection model.

What would we expect in terms of empirical patterns under each of these scenarios? If G-matrices impose only microevolutionary constraints and population/species eventually reach their single-adaptive peak, we would expect no particular relationship between the magnitude and direction of evolutionary change and its alignment with the G-matrix. We would also not expect any significant alignment of the species response to selection with the major axis of variation of the G-matrix. Evolution in this scenario would depend only on the position of the adaptive peak in relation to the population average. Alternatively, if G-matrices have enduring consequences at the macroevolutionary level by influencing the choice of peak, we would expect an association between the magnitude and direction of evolutionary change and its alignment with the major axis of the G-matrix (see Porto et al. (2015)). Furthermore, species diversification should be biased in the directions of highest variation in the G-matrix. Evolutionary change would depend not only on the position of the adaptive peaks in regard to the current position of the population averages but also on the G-matrix structure, which would affect the probability of reaching the various peaks (Fig. 6.3). A possible complication of the single peak model is one in which we have only one peak, but this single adaptive peak is not fixed, instead fluctuating randomly in the morphospace over time (Jones et al. 2012). What would be the expectations under this model? The answer would depend on the frequency and magnitude of the peak fluctuation over time, and on whether or not the G-matrix evolves. If peak displacements are small and rare, the expectations would be more in line with the transient constraints model. Conversely, if peak fluctuations are common or large in magnitude, it is possible that populations never quite reach equilibrium and traverse the morphospace walking on the line of least resistance, thus approaching the expectations from the rugged peak model.

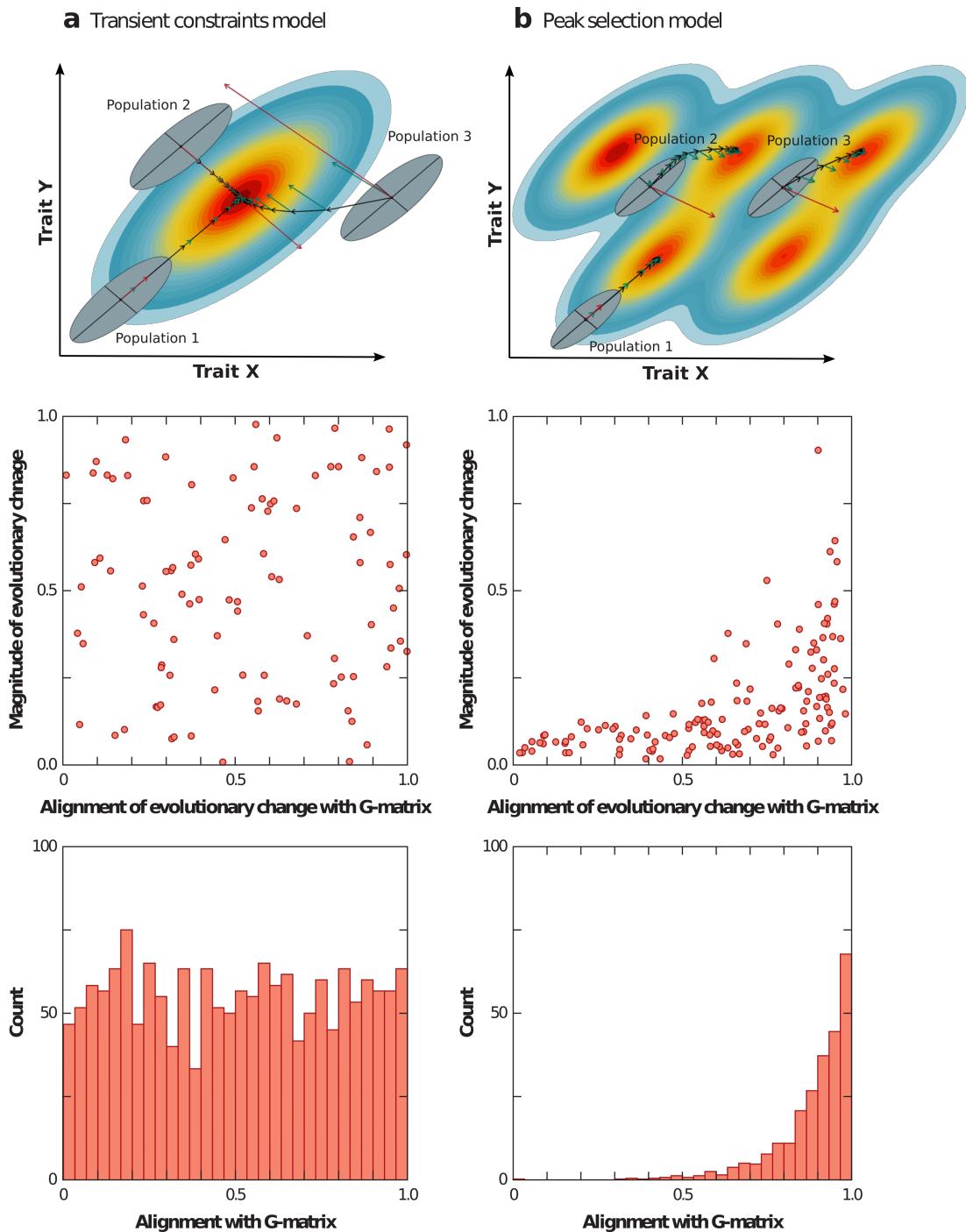


Figure 6.3: Left panels (a) show the transient constraints model, and right panels (b) show the peak selection model. Top left panel shows selection gradients (β) per generation (green arrows) and average responses (Δz) per generation (black arrows) of populations sharing similar G-matrix structure but at different starting points of a single-peaked adaptive landscape. Red arrows represent the net selection gradient (the sum of all selection gradients). Thus, the alignment between the direction of selection and the orientation of the G-matrix differs for each population. Responses to selection will thus vary between populations, in terms of direction and magnitude. Some populations will evolve rapidly and directly to the peak (*population 1*), whereas others will evolve slowly (*population 2*) owing to differences in the amount of variation aligned with selection (evolvability sensu Hansen and Houle (2008)) (*Continues*).

Figure 6.3: Population 3 will approach the peak in a nonlinear way, and its trajectory will be strongly deflected by the G-matrix in the direction of the line of least resistance. The line of least resistance equals the first principal component of a G-matrix and acts as an attractor of short-term evolutionary responses. We can expand this notion to multivariate systems and think of linear combinations of the first principal components as representing hyperplanes of least resistance. This notion is related to modularity, because principal components are related to modules but do not carry a one-to-one relation with each module (Berner et al. 2011). Usually, principal components are contrasts between modules (positive loadings for one module and negative loadings for the other), and linear combinations between these contrasts define directions of independent change for each module. Note that the net selection gradients (*red arrows*) are much larger when selection is not aligned with the G-matrix's main axis. The top right panel shows the same three populations but in a rugged adaptive landscape. In this scenario, populations won't always evolve to the closest peak (Euclidean distance) but instead will evolve to those that are closest given the covariation among traits. The central panels illustrate the predictions of each model (transient constraints, *left*; peak selection, *right*). Each point represents one species, with Y being the total magnitude of evolution, and X being the alignment of the evolutionary response (Δz) with the G-matrix. In the transient constraints model (*a*), you would not expect any particular relationship between the magnitude of evolutionary change and its direction, because every species would eventually reach the peak. Conversely, under the peak selection model (*b*), species' evolutionary trajectories may or may not be aligned with the G-matrix, but the magnitude of evolutionary change will be small when not aligned. Bottom panels show the predictions for both models (transient constraints, *left*; peak selection, *right*) of the number of species observed in terms of their evolutionary response (Δz) and their alignment with the G-matrix.

6.6.5 Does Alignment with Lines of Least Resistance Imply Constraint?

Comparisons of G-matrix orientation with the observed direction of evolutionary change, as described in the previous section, can be a fruitful way to test these ideas. Several studies have compared morphological diversification with available genetic variation: In several instances diversification was aligned with the lines of least resistance, whereas other instances showed diversification in alternate directions (Berner et al. 2010; Marroig and Cheverud 2010; Renaud et al. 2006; Schlüter 1996). But this alignment is not necessarily due to constraints, because selection and constraint can act in the same direction (Conner 2012; Marroig and Cheverud 2010). This would imply either that species lie near the axis of major evolvability not due to constraint but to a ridge in the fitness surface (Conner 2012) or that at least some of the available peaks happened to be aligned with that direction and thus the pattern is adaptive (Arnold et al. 2001; Marroig and Cheverud 2010). Likewise, macroevolutionary diversification that is not aligned with variation does not negate the possibility that the G-matrix imposed microevolutionary restrictions—it could be that the position of adaptive peaks had some other pattern. Perhaps a more complete picture of what we are observing is one close to the peak selection model. Species don't tend to follow the line of least resistance because they are constrained in that direction, in the sense of lacking variation in other directions of the morphospace (Marroig and Cheverud 2005, 2010). Instead, G-matrix and peak distribution interact, thereby making the realized morphospace coverage much smaller than the full range of possibilities. We now turn our attention to whether or not we can gain any information on past peak distribution from comparative quantitative genetic studies.

6.6.6 Differentiating Between Constraints, Co-Selection, and Drift

If the covariation between species is mirrored by the G-matrix, can we attribute this to constraints or to a common pattern of selection and covariation? Notice that examining the potential relationship between the evolutionary change and the divergence time between populations is not enough to separate constraints, selection, and drift. If changes among

populations are due to directional selection separating them into different adaptive peaks, and after the initial displacement their averages are kept constant by stabilizing selection, this sequence of directional and stabilizing selection may result in observed evolutionary rates that are consistent with drift (Lemos et al. 2001). Thus, information on the adaptive landscape and explicit tests for drift are necessary. So, can we examine the alignment of the orientation of the G-matrix with the distribution of peaks in the adaptive landscape? In theory, it should be possible to estimate covariation between selection in different clades (Fig. 6.4) on the basis of observed selection gradients, given some assumptions (Felsenstein 1988; Zeng 1988). Although this method gives us access only to the peaks that were eventually reached and are currently occupied by living species, it provides valuable information that can help us to explain whether macroevolution is dominated by constraints or by an interaction between constraints and selection, as in the peak selection model (Marroig and Cheverud 2010). Under transient constraints, we should not expect any alignment between the G-matrix and the selective covariance matrix (covariance between selection gradients), because, given enough time, the populations should eventually reach their respective adaptive peaks. Conversely, under peak selection, we would expect an alignment between the G-matrix and selective covariance matrix. We are aware of only two such tests reported to date (Hohenlohe and Arnold 2008; Marroig and Cheverud 2010).

Comparative approaches establishing a relationship between genetic lines of least resistance and divergence patterns have other important limitations. Most significantly, random genetic drift can create an association between the orientation of the G-matrix and the patterns of between-species divergence, given stable patterns of genetic covariation. This association occurs because evolutionary divergence under drift is expected to be proportional to the ancestral pattern of variation and covariation among traits (Fig. 6.2); therefore, an observed association between the orientation of the G-matrix and divergence can be a direct product of neutral evolution. Although most biologists would agree that morphology is usually under selection, it is useful to examine the potential consequences of drift and how it relates to modu-

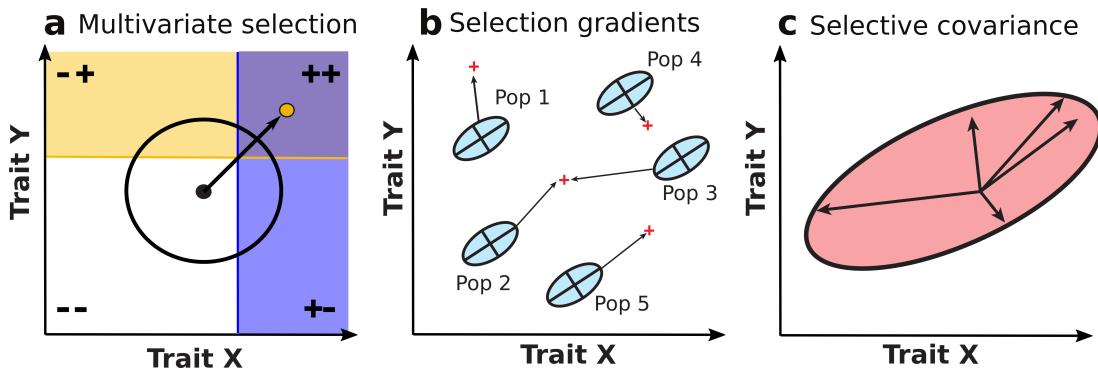


Figure 6.4: Traits will evolve together either because they are inherited together (G-matrix) or because they are selected together (selective covariance). Panel *a* illustrates the idea of selective covariance. Traits X and Y are genetically independent. The black dot indicates the average before selection, and the plus (+) and minus signs (-) indicate the direction of increase in fitness for each trait. Thus, selection is favoring the joint increase of X and Y, and the population will evolve a new average phenotype (yellow dot). The term selective covariance was coined by Felsenstein (1988) (see also Zeng (1988)). If we have evidence that the G-matrix is relatively stable during macroevolution, the equation $V = GCG$ captures the covariance of changes in the averages of the species (V-matrix) in terms of its two potential (non mutually excluding) sources: inheritance (G-matrix) and selective covariance (C-matrix). Theoretically, if we have a reasonable estimate of the G-matrix and of the phylogenetic relationships, we can compute the V-matrix and thus solve the Zeng-Felsenstein equation to compute $C = G^{-1}VG^{-1}$, where C is the covariance of slopes of $\log W$ (e.g., the covariance among the selection gradients operating upon each species). Panels *b* and *c* illustrate how to capture the matrix C with selection gradients (β) on panel *b* and the selective covariance matrix represented on panel *c* (pink ellipse). Different species are showed by letters a–e.).

larity. Simulation work suggests that if genetic drift is the only operating evolutionary process, modularity patterns would not be stable and patterns of association would vary widely across closely related populations or taxa (Jones et al. 2003; Melo and Marroig 2015). This is clearly not observed in nature. But what if modularity is maintained by stabilizing selection and trait means are free to change by genetic drift? In this situation, divergence among populations would be largest along directions in which ancestral genetic variation is abundant and smaller in directions of low ancestral variation (Arnold et al. 2001; Lande 1976). There are methods for distinguishing drift from selection in quantitative traits (Ackermann and Cheverud 2004; Bartoszek et al. 2012; Hohenlohe and Arnold 2008; Karhunen et al. 2013), but most of them are

not well suited to high dimensional systems, do not take the influence of genetic covariation into account, or require a large number of individuals distributed in a large number of species. For example, the approach from Hohenlohe and Arnold (2008) explicitly models evolution under drift to predict a probability distribution for the divergence of population averages, given a phylogeny, the G-matrix, and an estimate of effective population size. This elegant solution can test whether divergence among groups is compatible with drift and the current G-matrix, but it can be applied in full force only with two characters at a time. With few exceptions (Bartoszek et al. 2012; Hohenlohe and Arnold 2008), most phylogenetic methods fail to take genetic covariation into account, thereby limiting our understanding of macroevolution. By modeling evolution under a univariate Brownian motion model, for example, we assume that no selection is operating (but see Butler et al. (2004); Hansen (1997)) and that traits are evolving independently. Some advances have been made in the past decade (Bartoszek et al. 2012; Cressler et al. 2015; Hohenlohe and Arnold 2008), but we still lack comparative methods that balance the external aspect of selection (niche shifts on **Ornstein-Uhlenbeck** models, (Butler et al. 2004; Hansen 1997) with the populational consequences of modularity.

6.7 Conclusion

Placing micro- and macroevolution into a common framework is essential for our understanding of the influence of genetic and developmental constraints on multivariate evolution. Quantitative genetic theory has long been interested in the variational properties of organisms, and recent studies using the conceptual umbrella of modularity have extrapolated its breadth to include long-term evolutionary change. Although empirical results led us to discard the notion that variational patterns are set in stone and act as absolute constraints, they have also made us abandon the ideas that adaptive landscapes can be characterized by simple and stable selective peaks or that variational properties are largely unimportant considerations for evolutionary change. Embracing the dynamic nature of variational patterns, their context

dependency, as well as their relationships with genetics, development, and evolution will allow us to bridge these two levels of the hierarchy in a systematic way. One of the challenges going forward is the incorporation of mechanistic models of development into models of how variation emerges and how it influences the shape of population variation and adaptive landscapes. Another major challenge will be discriminating the relative contributions of constraints, selection, and neutral processes in determining the path of multivariate evolution. We propose that this challenge will be met only when we know more about the true shape of adaptive landscapes, including the number, height, and distribution of peaks (see Laughlin and Messier (2015); Pfaender et al. (2016)), and when we incorporate modularity into our thinking.

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Capítulo 7

Considerações Finais

Nesta tese abordamos o problema da evolução e manutenção dos padrões de covariância genética aditiva em caracteres complexos. Para isso, utilizamos seleção artificial em diversos desenhos experimentais. Todos os regimes de seleção artificial envolveram crescimento ou tamanho corporal. A complexidade da definição do tamanho de um organismo garante que seleção neste caráter terá influências indiretas em praticamente todos os caracteres morfológicos, e portanto as consequências da seleção dependem criticamente da arquitetura genética e do padrão de covariação. Neste capítulo final, vamos discutir brevemente os resultados de cada um dos três capítulos experimentais e tecer alguns comentários sobre as limitações e possíveis futuros desdobramentos do nosso trabalho.

A influência generalizada no organismo da seleção para alteração do tamanho corporal é bastante clara no capítulo 3. Neste capítulo, mostramos como seleção para alterações no peso, tanto para aumento quanto diminuição, causam respostas correlacionadas nos caracteres craniais. A alteração coordenada dos caracteres do crânio leva a uma mudança na sua estrutura da covariação genética e fenotípica. A mudança na covariação genética tem dois aspectos importantes. Primeiro, a quantidade de variação genética total diminui. Esse é o tipo de mudança esperado sob seleção de curto prazo em caracteres de base genética aditiva. A seleção provoca mudanças nas frequências alélicas dos loci envolvidos na variação dos caracteres sob seleção.

A medida que os alelos que conferem maior aptidão aumentam de frequência e eventualmente se fixam, a variância genética dos caracteres sob seleção diminui. Na seleção de caracteres multivariados, a predição clássica para a mudança da covariância sob seleção direcional é que o efeito seja o mesmo, com a redução da variação longo da direção sob seleção. O livro texto clássico Falconer e Mackay (1996) discute a mudança esperada na correlação entre dois caracteres selecionados na mesma direção. A expectativa nesse caso é que, ao final do processo seletivo, os alelos que influenciam os dois caracteres na mesma direção estejam fixados, e que portanto apenas alelos com efeitos na direção ortogonal à seleção estariam segregando em frequências intermediárias. Como só haveria variação na direção ortogonal aos dois caracteres mudando juntos, a correlação entre eles ao final do processo deveria ser negativa. Isso é o exato oposto do que nós observamos no crânio dos camundongos selecionados. A perda de variação total que nós observamos é completamente anisotrópica, e algumas direções perdem mais variação do que outras. Mas, ao contrário do esperado pelo modelo aditivo, a direção que perde menos variação é justamente a direção de seleção. Proporcionalmente à variação total, a seleção direcional aumenta a proporção de variação na direção de seleção. Nós interpretamos esse resultado à luz da teoria recente de genética quantitativa que inclui efeitos não aditivos na determinação do padrão de covariação (Cheverud 2001; Pavlicev et al. 2011). A inclusão de efeitos epistáticos no nosso modelo da evolução da covariância genética da conta das mudanças na covariância observadas nas linhagens selecionadas. Apesar disso, nossa estimativa da matriz de covariância genética é bastante deficiente devido à estrutura familiar das linhagens sob seleção, uma consequência do desenho experimental que não foi feito com esse tipo de medida em mente¹, e portanto boa parte dos resultados depende de algum nível de similaridade entre as matrizes genéticas e fenotípicas, que parece razoável dada nossa experiência com esses caracteres do crânio (Cheverud 1988; Garcia et al. 2014). Além disso, não temos nenhum dado de marcadores genéticos para esses animais, o que limita nossa habilidade de

¹O experimento de seleção artificial foi originalmente concebido em um departamento de ciências veterinárias, e o objetivo do estudo era avaliar a viabilidade das linhagens após seleção de longo prazo, com aplicações para melhoramento genético bovino. Mérito do Gabriel em perceber a possibilidade de aproveitar a colônia para o estudo da evolução da covariação.

confirmar a origem genética das mudanças na covariação. Mesmo com essas limitações, esses resultados ilustram bem o tipo de mudança bastante não intuitiva que seleção direcional pode ter na covariação genética e fenotípica num intervalo de tempo relativamente curto.

O capítulo 4 utiliza um cruzamento clássico (descrito em Cheverud et al. 1996) de camundongos, selecionados para alterações no tamanho corporal, para investigar o padrão pleiotrópico e a covariação genética entre fases do crescimento. Este capítulo tem dois temas principais: (1) a caracterização multivariada dos efeitos pleiotrópicos em relação à seleção e a divergência fenotípica entre os fundadores; (2) a inferência dos padrões de covariação a partir dos efeitos aditivos e de dominância. Cabe aqui um comentário sobre os dados utilizados nesse capítulo. Pelos padrões atuais, o número de marcadores genotípicos utilizado é bastante pequeno (cerca de 350 marcadores, 3 ordens de grandeza a menos que no capítulo 5). Essa baixa densidade, aliada às poucas gerações de recombinação a partir dos fundadores, limita a resolução dos nossos mapeamentos e a nossa habilidade de distinguir pleiotropia em sua definição mais estrita, de um locus com efeitos na variação de vários caracteres, de desequilíbrio de ligação entre loci não pleiotrópicos². Apesar disso, podemos pensar nos efeitos combinados de pleiotropia e desequilíbrio de ligação e quantificar como esses efeitos geram a covariação, sem tentar diferenciar os dois. Uma das vantagens de um banco de dados um pouco antigo e que já foi explorado de forma bastante completa na literatura (Cheverud et al. 1996; Hager et al. 2009; Kramer et al. 1998; Leamy et al. 2002; Mitteroecker et al. 2016; Vaughn et al. 1999; Wolf et al. 2005) é que nos sentimos mais livres para tentar métodos novos e explorar os dados de forma menos ortodoxa. Com isso em mente, nós utilizamos um modelo de mapeamento genético multivariado relativamente incomum, ajustando os efeitos aditivos e de dominância em todos os caracteres simultaneamente para cada marcador. Além disso, exploramos também uma regressão regularizada, que permite ajustar o modelo aditivo e de dominância para todos os marcadores simultaneamente.³ A partir dos vetores de efeitos aditivos e de dominância,

²Que eventualmente seria quebrado por recombinação

³Os resultados são semelhantes entre os dois métodos, e destacamos aqui que ajustar esse tipo de modelo sem utilizar programas escritos especialmente para o problema em questão seria impensável há alguns anos. Nós

nós mostramos como a divergência entre os fundadores se deve principalmente a efeitos aditivos, e, utilizando somente os vetores aditivos estimados na população recombinante, fomos capazes de prever de forma bastante satisfatória o fenótipo dos fundadores. Os vetores aditivos carregam o sinal de seleção, e vetores maiores são mais alinhados com a direção de seleção e divergência fenotípica entre fundadores. Em oposição aos efeitos aditivos, os efeitos de dominância não apresentam nenhum sinal de alinhamento com a seleção. Essa diferença provavelmente se deve em parte aos efeitos aditivos serem um resultado do processo seletivo que levou à diferenciação entre as linhagens fundadoras: os efeitos aditivos observados na F_3 são resultado da seleção, os efeitos de dominância não. Estes últimos são um fenômeno emergente do encontro de alelos que evoluíram separados, e o efeito de sua interação não foi moldado por seleção.

Além da diferença no alinhamento com seleção, os efeitos aditivos e de dominância também diferem na sua relação com o padrão modular. Como os efeitos aditivos são mais alinhados com seleção, poderíamos esperar que a distribuição de efeito aditivos tivesse o primeiro componente de variação entre efeitos alinhado com a seleção. Apesar disso, a distribuição de efeitos aditivos recupera o padrão modular entre as fases precoce e tardia do crescimento, com as duas principais direções de variação dos efeitos aditivos alinhadas com as duas fases do crescimento. Uma hipótese que poderíamos levantar para explicar esse padrão modular é uma influência do desenvolvimento na expressão dos efeitos aditivos, que de alguma forma poderia restringir o padrão de pleiotropia. Mas, se esse fosse o caso, a mesma restrição também se aplicaria aos efeitos de dominância. Mas a distribuição dos efeitos de dominância não apresenta nenhum padrão modular. Aqui, com nossos dados, só podemos especular, mas podemos novamente pensar na relação entre a história dos efeitos aditivos, que são moldados por seleção, e o processo de desenvolvimento, que difere nas fases precoce e tardia do crescimento. Juntas essas duas influências podem explicar a diferença entre os efeitos aditivos e de dominância, pois o

implementamos todos os modelos em programas genéricos para o ajuste de modelos lineares. Esse processo em si foi um aprendizado, e nosso sucesso mostra o quanto poderosas são as ferramentas estatísticas que temos disponíveis hoje.

efeito da seleção precisa de alguma forma ser processado pelo desenvolvimento (Klingenberg 2008). Ao mesmo tempo, os efeitos de dominância são expressos pelo desenvolvimento, mas de forma emergente e menos relacionada com a história evolutiva da população.

A relação entre os efeitos pleiotrópicos e a modularidade é explorada de forma direta, e nós estimamos a matriz de covariância esperada dados os efeitos pleiotrópicos detectados pelo mapeamento genético. Essa matriz esperada é bastante similar à matriz genética estimada pela similaridade entre irmãos completos, e nós mostramos como as matrizes devido aos efeitos aditivos e de dominância tem padrões modulares diferentes, reforçando o efeito na população das diferenças exploradas acima. A combinação desses efeitos distintos forma a covariância observada na F_3 .

O estudo da ligação entre efeitos pleiotrópicos e covariação é continuada no capítulo 5, que funciona como uma extensão do capítulo 4, mas que procura abordar o mesmo problema de outra perspectiva. Esse capítulo utiliza um banco de dados mais moderno, de 33300 marcadores e uma geração mais avançada de um intercruzamento de camundongos mais complexo. Esse intercruzamento foi gerado utilizando fundadores vindos de um experimento de seleção artificial para a alteração da curva de crescimento, com seleção divergente sendo aplicada nas fases precoce e tardia. O resultado deste regime seletivo é uma mudança generalizada na taxa de crescimento ao longo de todo o desenvolvimento dos animais. A mistura de seis linhagens com curvas de crescimento muito divergentes gerou uma população ultra variável em sua curva de crescimento, e que apresenta um padrão de covariação entre as fases do crescimento bastante diferente do padrão ancestral, antes da seleção. A estrutura familiar mais complexa desses dados cria algumas dificuldades metodológicas no mapeamento genético, pois para levar o efeito do parentesco entre indivíduos em conta no mapeamento genético é necessário estimar um modelo animal para cada marcador. Existem métodos aproximados bastante eficientes para a realização desse mapeamento, como o FaST-LMM (Lippert et al. 2011) ou o GEMMA (Zhou e Stephens 2012), mas a maioria desses programas se limita a estimativa de efeitos aditivos, ignorando os efeitos de dominância. Neste capítulo, por restrições

de tempo e por essa restrição do programas eficientes de mapeamento, nos limitamos a explorar a contribuição aditiva dos efeitos pleiotrópicos para a covariância. Além da população experimental, nós também desenvolvemos um modelo de simulação computacional para a evolução de caracteres quantitativos controlados por genes pleiotrópicos, no qual o padrão de pleiotropia pode evoluir de forma contínua. A partir deste modelo, criamos algumas expectativas qualitativas para a distribuição de efeitos pleiotrópicos em diversos cenários de seleção estabilizadora e direcional. Essas expectativas são baseadas na classificação dos vetores de efeitos pleiotrópicos em categorias de acordo a direção do efeito em relação ao um padrão modular pré-definido. As simulações sugerem que no cenário de seleção divergente, como foi o caso dos fundadores da população intercruzada, haveria um aumento na proporção de alelos que afetam as duas fases do crescimento em direções opostas, um padrão pleiotrópico que nós chamamos de antagonista. Esses efeitos antagonistas seriam os principais responsáveis pelas correlações positivas dentro de cada fase do crescimento e pelas correlações negativas entre as fases. De fato, no mapeamento de loci quantitativos na geração F_6 do intercruzamento, encontramos uma série de efeitos antagonistas, mas, diferente da simulações, não mais do que as outras classes de efeitos, modulares, integrativos e intra-modulares. A matriz de correlação genética esperada dados todos os efeitos que conseguimos detectar é relativamente parecida com a matriz de covariância genética estimada a partir de um modelo animal. Apesar disso, as matrizes esperadas para cada classe de efeito pleiotrópico não é compatível com a nossa expectativa ao criar a classe. Por exemplo, os efeitos modulares, em tese, deveriam contribuir para a correlação dentro de módulos, mas a matriz esperada a partir dos efeitos modulares apresenta correlações altas entre módulos. Da mesma forma, os efeitos integradores deveriam gerar correlações positivas entre todos os caracteres, mas a matriz esperada a partir desses efeitos apresenta correlações negativas. A coerência do padrão global e os problemas dos padrões dentro de cada classe sugere que nossas estimativas de cada um dos efeitos pleiotrópicos é ruidosa demais para que a classificação seja feita de forma confiável, mas o padrão global recupera algum sinal biológico. Nossa habilidade de caracterizar as origens do padrão de cova-

rião observado também foi prejudicada pela ausência do componentes de dominância, que, especialmente nessa população, tem um grande potencial de contribuir para a covariância⁴. Apesar dessa deficiência, o estudo dos efeitos aditivos ilustra como o padrão de covariância é formado por contribuições de diversos tipos de efeitos pleiotrópicos que interagem de forma complexa.

Próximos passos

As análises dos capítulos 4 e 5 se limitam a caracterizar a distribuição de efeitos pleiotrópicos presentes nas populações intercruzadas. Essa distribuição é interpretada à luz das nossas expectativas teóricas e relacionada à história evolutiva dos fundadores das duas populações. Os vetores de efeitos pleiotrópicos são também relacionados ao padrão de covariação entre os caracteres. Essa caracterização fina da distribuição de efeitos pleiotrópicos nos informa sobre as origens da covariação genética, e da pistas sobre a distribuição de efeitos mutacionais e sobre os processos de desenvolvimento que dão origem a esses efeitos pleiotrópicos. O que nossa análise não fornece é uma quantificação da variação nesses efeitos. Para isso, seria necessária a inclusão de efeitos epistáticos nos modelos de mapeamento, e essa é uma extensão fundamental de toda nossa análise. Animais vindos do mesmo cruzamento de camundongos utilizado do capítulo 4 já foram usados para o estudo do efeito de interações gênicas em diversos contextos, como uma quantificação da contribuição de interações epistáticas na relação alométrica entre ossos longos e peso (Pavlicev et al. 2008), para avaliar a contribuição de epistasia para a covariância entre caracteres cranianos que se desenvolvem em fases diferentes do desenvolvimento (Wolf et al. 2005), e para quantificar variação na intensidade das correlações entre caracteres mandibulares (Cheverud et al. 2004). Esses exemplos ilustram a contribuição importante dos efeitos epistáticos para a covariação aditiva, e, principalmente, a contribuição dos efeitos epistáticos na variação dos efeitos pleiotrópicos.

⁴Isso porque a estrutura dos cruzamentos garante que um alelo presente em um dos fundadores vai estar em frequência próxima de 1/6 na geração F₆, e quanto mais longe da frequência de 1/2, mais o componente dominante contribui para a covariação aditiva.

Para dar continuidade ao estudo da evolução da covariação, pretendemos investigar a relação entre os efeitos epistáticos e a história seletiva das linhagens em um conjunto mais diverso de caracteres. Na população intercruzada do capítulo 5, que foi criada a partir de seis linhagens fundadoras, nós também medimos uma série de fenótipos ligados à composição corporal e ao sistema esquelético como um todo, incluindo medidas de alta resolução do crânio. Todos esses fenótipos são divergentes entre as linhagens fundadoras, e de formas mais complicadas que simplesmente diferenças em tamanho corporal⁵. Uma vantagem de ampliar a análise para outros fenótipos é que estes podem ser cada vez mais afastados do alvo de seleção. Aqui nós analisamos a arquitetura genética dos caracteres sob seleção, e isso tem consequências para o padrão de efeitos pleiotrópicos, como vimos principalmente no capítulo 4. Quando nos afastamos do alvo de seleção, ainda observamos divergência, mas essa divergência pode potencialmente ser menos estruturada pela seleção e fornecer padrões diferentes de epistasia e pleiotropia. Como a variação de efeitos pleiotrópicos depende das interações epistáticas, essas diferenças trazem consequências para a evolução da covariância entre caracteres, como foi discutido brevemente no capítulo 3. Estudar a variação epistática em conjuntos de caracteres cada vez mais distantes do alvo de seleção pode potencialmente trazer uma gama maior de organizações pleiotrópicas e de padrões de covariação.

O estudo de caracteres cranianos na população do capítulo 5 também fornece um sistema controlado para entender como as mudanças no padrão de integração observados no crânios do capítulo 3 efetivamente acontecem ao nível de efeitos pleiotrópicos. Os resultados em Porto et al. (2016) sugerem que essas mudanças são devido ao número de alelos pleiotrópicos de efeito generalizado, e a escala de tempo em que nós observamos as mudanças de integração sugerem qualquer mudança nos efeitos pleiotrópicos deve ser baseada em variação epistática, pois não haveria tempo de acumular mutações pleiotrópicas suficientes para explicar a mudança de integração observada no capítulo 3. Além disso, a população intercruzada do capítulo 5

⁵Os cruzamentos entre as linhagens *Large* e *Small*, utilizados no capítulo 4 e por boa parte da literatura em efeitos genéticos e covariação, sofrem do problema da simplicidade da divergência entre os fundadores. É difícil investigar padrões mais elaborados de pleiotropia na morfologia se a maioria dos efeitos está alinhado com a divergência em tamanho e a maioria dos efeitos pleiotrópicos é sinérgico.

apresenta uma curva de crescimento muito variável, e portanto se presta ao estudo da relação entre integração no crânio e taxa de crescimento, dois caracteres relacionados por um padrão macroevolutivo claro (Porto et al. 2013). Esse padrão macroevolutivo associa taxas de crescimento (ou investimento energético no crescimento) elevado com taxas de integração elevadas em diversos táxons. Nossa banco de dados permite verificar se esse padrão macroevolutivo se mantém no nível microevolutivo e tentar entender os processos que levam a essa associação.

Um resultado consistente ao longo de todos os capítulos é a presença de variação no padrão dos efeitos pleiotrópicos e, consequentemente, a habilidade do padrão de covariação de mudar em resposta a pressões seletivas. A intensidade das covariâncias muda de forma bastante rápida sob seleção no experimento do capítulo 3, e o padrão de correlação foi alterado por seleção nos fundadores do capítulo 5. Os efeitos pleiotrópicos detectados nos capítulos 4 e 5 podem potencialmente gerar padrões de covariância diferentes somente com alterações das frequências alélicas, mesmo quando ignoramos as interações epistáticas. Essa maleabilidade dos padrões de covariância era sugerida por modelos teóricos (Arnold et al. 2008; Barton e Turelli 1989), e realmente nós temos uma literatura considerável documentando mudanças significativas no padrão de covariância de diversos tipos de caracteres medidos em populações naturais, numa escala de tempo ecológica (Björklund et al. 2013; Doroszuk et al. 2008; Eroukhmanoff e Svensson 2011; Merilä e Gustafsson 1996; Pfrender e Lynch 2000), e até em escala de tempo macroevolutivo, associadas a mudanças de hábito locomotor (Young e Hallgrímsson 2005; Young et al. 2010). Existem alguns problemas metodológicos com esse tipo de estudo em populações naturais, principalmente devido à dificuldade em se obter amostras grandes o suficiente aliada à dificuldade inerente em se estimar uma matriz de covariância genética de forma suficientemente precisa (Marroig et al. 2012). Devido a esses problemas de estimativa, é bastante difícil diferenciar mudanças biologicamente relevantes de mudanças significativas mas irrelevantes, principalmente porque a maioria dos métodos de estimativa da matriz de covariância genética aditiva não fornecem boas estimativas do intervalo de confiança para as

covariâncias⁶ e métodos eficientes para estimar esses intervalos são relativamente incipientes (Houle e Meyer 2015; Runcie e Mukherjee 2013). De qualquer forma, todos esses resultados apontam para um quadro de mudanças rápidas de covariação e de variação abundante para o padrão e intensidade de covariâncias. Por outro lado, varias estimativas de padrões de covariância estimados em populações naturais apresentam uma notável estabilidade numa escala macroevolutiva (Garant et al. 2008; Marroig e Cheverud 2001; McGloethlin et al. 2018; Porto et al. 2009; Steppan 1997) mesmo com a facilidade observada em se modificar o padrão de covariância em curto prazo. A questão da estabilidade macroevolutiva da matriz de covariância genética é fundamental para o estudo de diversificação, pois uma matriz G estável permite reconstruir o padrão de seleção ancestral (Jones et al. 2004) e implica uma possível restrição macroevolutiva à resposta seletiva, principalmente em superfícies adaptativas com muitos picos, como discutimos no capítulo 6. Essa inconsistência entre resultados de curto e longo prazo sugere que existem forças evolutivas mantendo o padrão de covariância estável em escalas de tempo longas, e algumas propostas foram feitas nesse sentido, como estabilidade dos padrões gerais de alocação de recursos (Björklund 2004), ou processos de seleção estabilizadora interna (Cheverud 1984), relacionados à compatibilidade entre caracteres dentro do mesmo organismo. Outro mecanismo possível para a manutenção da matriz G em longas escalas de tempo é a constância da matriz de covariância mutacional, pois mesmo que flutuações de curto prazo alterem a matriz G, novas mutações podem reestabelecer o padrão variacional. Existe alguma evidência para um alinhamento entre matrizes mutacionais e genéticas (Houle et al. 2017), mas ainda não sabemos como esse mecanismo para a manutenção da covariações se comporta sob seleção e como ele se manifesta no padrão de efeitos pleiotrópicos. Esperamos dar continuidade no estudo da evolução das covariações genéticas nesse contexto macroevolutivo, e entender os mecanismos que regem a interação entre restrições genéticas e os processos evolutivos.

⁶As aproximações envolvidas em calcular o erro padrão de um parâmetro de variância num modelo animal ajustado por máxima verossimilhança são bastante duvidosas, como por exemplo a simetria da distribuição do parâmetro. Principalmente no caso relativamente comum de um parâmetro ser estimado na borda do seu intervalo de suporte, em que a distribuição do parâmetro é completamente assimétrica.

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