Effects of pleiotropy on the response to selection:

Achieving evolvability in a simulated struggle for existence

Thomas Mørtvedt Solvin

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Centre for Ecological and Evolutionary Synthesis (CEES)
Department of Biology
Faculty of Mathematics and Natural Sciences

University of Oslo

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http://www.duo.uio.no/

Abstract

The independent evolution of different quantitative traits is often thought to require a modular structure of the genotype-phenotype map (GP map). In that context, pleiotropy is considered a constraint on adaptive evolution. Previous studies have shown that even though a pleiotropic GP map can avoid unfavorable genetic correlations among traits, pleiotropy still impedes evolution across multiple generations. In this study, a linear model of the GP map is used to investigate the effects of pleiotropy on the evolvability of quantitative traits. An R script is made for population simulations of two quantitative traits under conflicting selection pressures, by means of which a variety of GP maps of different levels and types of pleiotropy are compared, both mutually and with modular GP maps. In addition, the predictive power of quantitative genetic measures of evolvability is tested. The results show that GP maps with extensive pleiotropy can be equally optimal as modular ones, implying that evolvability does not require modularity. Examples are provided both where pleiotropy constrains and where it enhances the response to selection, depending on underlying assumptions of the GP map. It is further shown that quantitative genetics theory can accurately measure evolvability, even when genetic correlations and conflicting selection pressures are present. In addition, other properties of the GP map affecting evolutionary response are considered.

IV

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Contents

1 Introduction

The goal of this project is to make a contribution towards better understanding of how the genetic architecture affects the evolution of quantitative traits. Several studies have investigated how genetic correlations and evolvability are affected by the genetic architecture (Lande 1980; Cheverud 1984; Wagner 1989; Slatkin and Frank 1990; Houle 1991; Gromko 1995; Wagner and Altenberg 1996; Baatz and Wagner 1997; Hansen 2003; Griswold 2006; Walsh and Blows 2009). However, basic questions about how standing genetic variance is molded by the pleiotropy structure of the genotype-phenotype map, and thereby rendered available or not for selection response, seem to remain unresolved. This is true particularly for evolution across multiple generations through which predictions from analytical theory cannot reach (Lande and Arnold 1983; Arnold et al. 2008). What genetic architecture optimizes the response to directional selection? What is the optimal level of pleiotropy? How well can the evolutionary response be predicted under different genetic architectures? This study is meant to shed some light on these and other issues involving evolutionary response as a function of pleiotropy.

I have investigated how different structures of the genotype-phenotype map (GP map) with respect to pleiotropy affect character evolvability. Pleiotropy is regarded both as a feature facilitating coordinated evolution of functionally related characters through integration, and as a major source of genetic covariance constraining independent character evolution. It has been suggested that evolvability is obtained by modularity of the GP map (Wagner and Altenberg 1996; Kirschner and Gerhart 1998). Modularity has been found in several traits at different phenotypic levels and in different groups of organisms (Wagner et al. 2007). This is however not the only way the GP map can be structured to avoid constraining genetic covariances (Mitteroecker 2009), and evolvability does not necessarily require modularity (Hansen 2003). Another possible genetic architecture that avoids genetic correlations is a GP map where the pleiotropy is "hidden".

The rate of evolution of a quantitative trait is determined by the strength of selection and the level of underlying additive genetic variance (evolvability). Pleiotropy can however tie up parts of this variance by linking it to other traits that are under conflicting selection regimes. The rate of evolution is then determined by the remaining free genetic variance (conditional evolvability (Hansen 2003)). Pleiotropy is thus generally regarded as a constraint on

evolvability (Griswold 2006), and modularity is seen as a way of avoiding such constraints (Wagner and Altenberg 1996). By means of stochastic population simulations, I have tested the hypothesis that pleiotropy reduces evolvability even when it his hidden, that is even when it is not generating genetic covariances. Studies have shown that this can be the case under some conditions, but that pleiotropy also can increase evolvability under different conditions (Baatz and Wagner 1997; Hansen 2003; Griswold 2006). I further test the hypothesis that pleiotropy can enhance evolvability by acting as a source of variation even when this ties some of the variation up with a trait under conflicting selection. In that respect I seek to estimate the optimal level of pleiotropy (Hansen 2003). Another question I address regarding effects of the genetic architecture on evolution is, whether the number of loci underlying a trait with a certain level of additive genetic variance is important. I hypothesize that this can be important, and should be considered when studying the genotype-phenotype map. In addition I test how well the conditional evolvability of Hansen (2003) predicts evolution across multiple generations, and whether its predictive value depends on the underlying GP map. This measure is based on the multivariate Lande equation, whose predictive value needs testing (Roff 2007).

This is thus a thesis in evolutionary quantitative genetics, a field within evolutionary biology that addresses quantitative traits. These are phenotypic traits that vary continuously and that are typically underlain by many loci (Falconer 1981). If we consider the effect of one locus as a random variable, and assume random mating and additivity of effects among loci, thereby disregarding epistasis and dominance effects, the distribution of a quantitative trait will be approximately normal (Bulmer 1980). There are nonetheless also other effects a trait is subject to and the total phenotypic variance of a trait (V_P) can be partitioned into genetic variance (V_G) and environmental variance (V_E). Genetic variance can further be divided into additive genetic (V_A), dominance (V_D) and epistatic (V_I) variance (Falconer 1981). Dominance variance comes from the interactions between the alleles at the same locus, whereas epistatic variance results from interactions between alleles at different loci. Environmental variance arises from among-individual variation in the environment, that is, the different individuals in a population experience different environments. Although it is shown that epistasis can potentially have dramatic effects on the response to selection, the general view is that the additive genetic variance is the evolutionary important component (Hansen 2006; Roff 2007).

2 Theory

2.1 Evolvability

The concept of evolvability, meaning the ability to evolve, is central in quantitative genetics. Why do organisms evolve, and what is needed for adaptive evolution? As a starting point, these questions can be answered by simple inspection of the Lande equation.

Consider a vector $\boldsymbol{z}$, where each entry z_k represents the size of a different quantitative trait in an organism. Assume $\boldsymbol{z}$ equals the sum of additive genetic ($\boldsymbol{x}$) and environmental effects ($\boldsymbol{e}$), $\boldsymbol{z} = \boldsymbol{x} + \boldsymbol{e}$, where $\boldsymbol{x}$ and $\boldsymbol{e}$ are independent and multivariate normally distributed. Lande (1979) then shows that the multiple trait response ($\Delta\bar{\boldsymbol{z}}$) from one generation to the next is given by:

$$\Delta\bar{\boldsymbol{z}} = \boldsymbol{G} \, \nabla \, ln\,\bar{W}.$$

The term $\nabla \ln \bar{W}$ is defined as the *selection gradient*, where $\nabla = \begin{bmatrix} \frac{\partial}{\partial\bar{z}_1} \\ \vdots \\ \frac{\partial}{\partial\bar{z}_n} \end{bmatrix}$, n is the number of traits, $\bar{z}_k$ is the population mean of trait k and $\bar{W}$ is the mean population fitness. Its elements give the change in fitness per change in each single trait. Alternatively, the selection gradient can be written as $\boldsymbol{\beta}$, defined as the vector of partial regression coefficients of individual relative fitness (w) on the characters (Lande and Arnold 1983), and the Lande equation becomes:

$$\Delta\bar{\boldsymbol{z}} = \boldsymbol{G} \, \boldsymbol{\beta}. \tag{1}$$

The vector $\Delta\bar{\boldsymbol{z}}$ gives the change in population mean of each trait from one generation to the next, and $\boldsymbol{G}$ is the additive genetic variance-covariance matrix (the G matrix):

$$\Delta\begin{bmatrix} \bar{z}_1 \\ \bar{z}_2 \\ \vdots \\ \bar{z}_n \end{bmatrix} = \begin{bmatrix} G_{1,1} & G_{1,2} & \cdots & G_{1,n} \\ G_{2,1} & G_{2,2} & \cdots & G_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ G_{n,1} & G_{n,2} & \cdots & G_{n,n} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_n \end{bmatrix}.$$

The term $\Delta \bar{z}_k$ is the change in mean value of trait k, β_k is the change in mean fitness per change in population mean of trait k, $G_{k,h}$ is the additive genetic covariance between trait k and h. If h equals k, $G_{k,h}$ is the covariance of the trait with itself, which is the additive genetic variance of trait k (V_{Ak}). The unit of β_k is one divided by the unit of the respective trait. For a single trait, the equation becomes $\Delta \bar{z} = V_A \beta$.

If we define evolvability as the ability to respond to selection, we see that when $\Delta \bar{z} = V_A \beta$, this property lies within the additive genetic variance (V_A). The Lande equation separates selection from evolvability, which is essential when using this definition of evolvability (Hansen et al. 2003b). As Lande explains, the response in multiple traits ($\Delta \mathbf{z}$) does not necessarily go in the direction of maximal fitness increase ($\boldsymbol{\beta}$). It is dependent on the structure of the additive genetic variance-covariance matrix $\mathbf{G}$, and as the Lande equation shows, the response will be equal to the product of $\mathbf{G}$ and $\boldsymbol{\beta}$ (or $\nabla \ln \overline{W}$). However, a fitness peak will eventually be attained if the adaptive landscape remains constant and there is some level of additive genetic variation in all dimensions. This places the evolvability in the G matrix, and a measure of evolvability of a single character is simply the additive genetic variance of that character (V_A), or if comparisons with other characters are to be made, the additive genetic variance standardized by the trait mean, $\frac{V_A}{\bar{z}^2} = I_A$ (Houle 1992), (Hansen et al. 2003b).

The ability to respond to selection, or evolvability, thus corresponds to the amount of additive genetic variance V_A. Based on this, how evolvable do we expect natural populations to be, and how high evolutionary rates do we expect to observe? Lynch (1990) investigated how fast morphological traits in mammals have evolved compared to what is expected based on neutral evolution, that is, if there was no selection, and evolution was driven only by mutation and genetic drift. His main result was that the rate of morphological divergence of mammalian groups is generally well below the neutral expectation. Most quantitative characters should actually be evolvable, as they generally possess large amounts of V_A (Hansen and Houle 2004; Arnold et al. 2008). The challenge in evolutionary quantitative genetics today is therefore not to explain rapid evolutionary change, but rather the lack of it, the stasis.

2.2   Constraints and Pleiotropy

In quantitative genetics the frequently observed stasis has been difficult to explain. The amount of genetic variation does not seem to constrain adaptive evolution (Futuyma 2010),

and most traits exhibiting stasis appear to be genetically variable (Hansen and Houle 2004). Stabilizing selection has been invoked as explanation for the low rate of morphological evolution in mammals (Lynch 1990). However, for stabilizing selection to maintain stasis the selective optimum must be constant, and a satisfactory explanation for it to be so has not been made (Hansen and Houle 2004).

One explanation for the existence of stasis is due to entanglement of the character's variance with other characters. When constrained by other characters under stabilizing selection, the character's evolvability may be reduced, as expressed in so-called conditional evolvability (Hansen et al. 2003a). Walsh and Blows (2009) argue that the apparent paradox can be resolved by considering the geometry of the variation (and selection) in multiple traits. They explain that although selection should deplete genetic variance, this may not be observable from a single character perspective, as the direction of selection may be different from the axes of high genetic variation in phenotype space. Thus, while the individual characters may have substantial levels of additive genetic variance, because of genetic covariances between them evolution may not be able to move in the direction of natural selection. This can be deduced from the Lande equation: For a two-locus scenario the response of a character z_1 is determined by the directional selective strength on that character β_1 times its additive genetic variance (V_{A1} or $G_{1,1}$). However, it may also be affected by the selection on another character z_2 if there is additive genetic covariance between them:

$$\Delta \bar{z}_1 = G_{1,1}\, \beta_1 + G_{1,2}\beta_2. \tag{2}$$

That is, selection on trait z_2 (β_2) induces a correlated response in trait z_1 ($G_{1,2}\beta_2$). Consider the case where we want to change trait z_2 without changing trait z_1. This is equivalent to directional selection on trait z_2 combined with stabilizing selection on trait z_1. Due to opposing selective forces the change in trait z_2 may be impaired (fig. 1). The two phenomena generating such genetic covariances are linkage disequilibrium (LD) and pleiotropy (Lande 1980). The evolutionary important one however is pleiotropy, as recombination eliminates covariances due to LD. Lande (1980) explains that LD contributes only little to genetic covariances when selection is weak relative to recombination rate. In my simulation experiments there is free recombination among loci, so I generally disregard LD as a significant factor affecting evolutionary response. However, I did test this assumption (section 3.2.8).
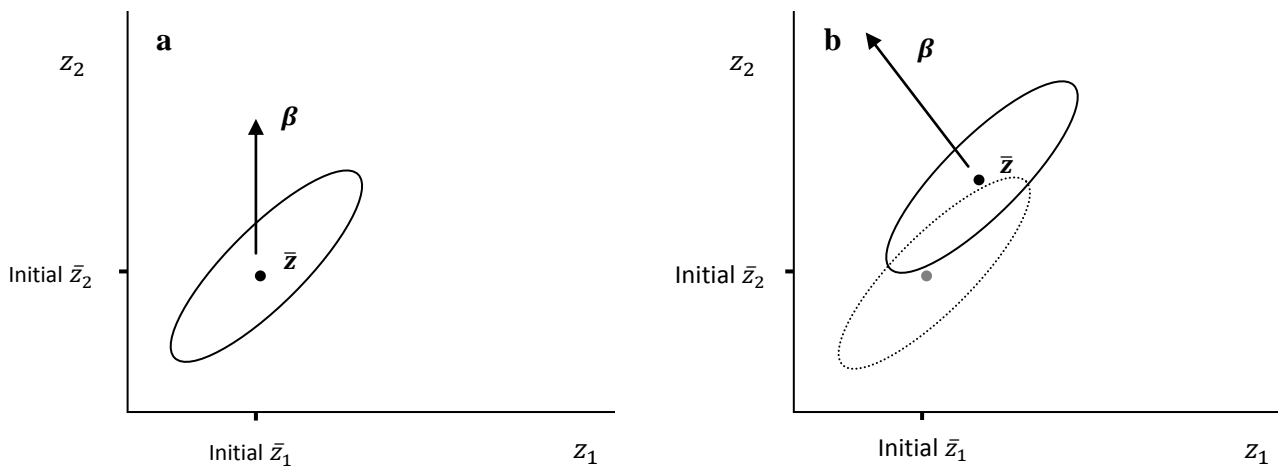
Figure 1 – a) Initially the population, whose phenotypic distribution is represented by the ellipse, is subject to positive directional selection on z_2 while z_1 is at its optimum. b) Because of positive genetic covariance between the two traits there is a positive response in both, and the population mean is displaced from the grey dot to the black one. In addition to directional selection on z_2, the displacement induces selection for bringing z_1 back to its optimum resulting in a net selection gradient (β) prependicular to the major axis of the phenotypic distribution. As there is little genetic variance in this direction, evolution is slowed down.

Pleiotropy means that a gene affects two or more characters (Falconer 1981). The term was first used in 1910 by the German geneticist Ludwig Plate. The concept was, however, in some sense known prior to this, as the observation that multiple medical symptoms could have a single common factor was recognized already in pre-Mendelian times (Stearns 2010). According to Plate's definition "a unit of inheritance" is pleiotropic if several characteristics are dependent upon it. Pleiotropy is expected to be abundant as it has certain evolutionary advantages. When an adaptive change requires coordinated changes in multiple traits, fewer mutations are needed if a mutation affects several rather than one trait, thus increasing the probability of adaptation (Cheverud 1984). This is achieved by pleiotropy, as modification of one gene may induce changes in several characters, reducing the number of mutations needed. However, pleiotropy also has the disadvantage of constraining evolution when correlated characters are under conflicting selective forces as shown above. This could easily occur if pleiotropy was abundant between functionally independent characters such that directional selection on one of them was unlikely to be associated with directional selection on the other, which would, as most traits, expected to be under stabilizing selection, assuming they track local selective optima (Hansen and Houle 2004; Walsh and Blows 2009).

6

The concept of "cost of complexity" states that the predicted rate of adaptation decreases with increasing number of traits underlying an organism's phenotype ("complexity") (Wagner and Zhang 2011). The reason for this is explained by a model of the scientist who initiated these ideas, Fisher (1930). He presents the phenotype of an organism as a point A in space some distance R from its optimum O, the number of dimensions of the space representing the number of underlying traits n. For a random change of the phenotype moving A a distance r, an adaptation is present if A ends up closer to O than it was before. For this to be possible r must be smaller than $2R$, and the probability of adaptation will tend to one half for an infinitely small r. However, for an r in between these values, the probability of adaptation decreases with increasing n. This can be visualized by comparing phenotypes of one, two and three dimensions. In one dimension, the phenotype A lies on a line at a distance R from O, thus it can only move in two directions. For $0 < r < 2R$ the probability of improvement will always be one half. In two dimensions however, the phenotype can move in any direction in a plane, ending up somewhere on the circle with center A and radius r. The probability of improvement is represented by the part of this circle lying within the other circle with center O and radius R (fig. 2). For $0 < r < 2R$, this will equal $arccos(r/2R)/\pi$, which is smaller than one half. In three dimensions, the probability of improvement equals $0.5 \cdot (1 - r/2R)$, which is even smaller on the interval $[0,2R]$ (fig. 3).
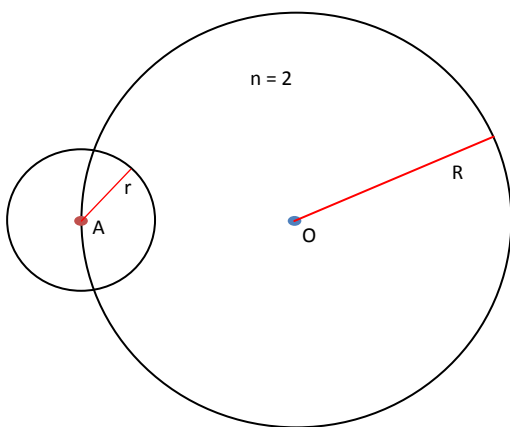
Figure 2 - **For complexity level $n = 2$ the probability of a phenotypic change of size r of being adaptive equals the fraction of the circle with radius r that is inside the circle with radius R, as moving the current phenotype A to a point on this part of the circle brings it closer to the optimum O.**
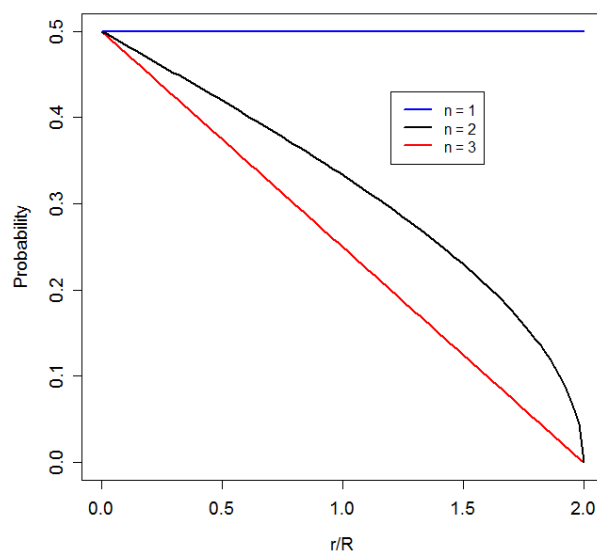
Figure 3 - The probability of adaptation as a function of size of phenotypic change (r) relative to phenotypic distance from optimum (R) for different levels of complexity (n).

7

According to Fisher's geometrical model (Fisher 1930), for large values of n, the probability that a random change of size r is adaptive equals $P(adaptation) = P(T \geq x)$, $T \sim N(0,1)$, $x = \frac{r * \sqrt{n}}{2R} \geq 0$. This probability decreases when n increases. According to Orr (2000) the value of x that maximizes $\frac{d\bar{w}}{dt}$ is 0.925. Kimura (1983), however, took into account that mutations with higher adaptive advantage have higher probability of fixation, and showed that adaptive evolution is most rapid when mutations have intermediate effects. Assuming mutations of constant size r Orr (2000) derived that $\frac{d\bar{w}}{dt}$ as a function of n declines faster than $\frac{1}{n}$ ($\bar{w}$ being fitness), thus there seem to be a high cost of complexity. As this model allows for universal pleiotropy, where any mutation may affect any character (Orr 2000), the complexity n can be considered the degree of pleiotropy and the cost of complexity is thus actually a cost of pleiotropy (Wagner and Zhang 2011). The assumption of constant mutational size regardless of pleiotropic effects seems however not to be justified empirically (cf. next section).

The cost of pleiotropy will surely be immense in a complex organism with a great number of traits. If a mutation at any locus can affect all traits, the probability of such a mutation being beneficial will drop to almost zero and adaptive evolution will be rendered impossible. As pleiotropy is a common phenomenon and up until recently has practically been regarded as universal (Stearns 2010; Wagner and Zhang 2011), it is seen as an important constraint to adaptive evolution.

2.3   Genotype-phenotype map

The genotype-phenotype map (GP map) describes how genetic variation is translated into phenotypic variation (Wagner and Altenberg 1996), by e.g. describing the number of loci per trait and the distribution of genetic effects across loci and traits. Pleiotropy is a very important aspect of the GP map. One familiar case of specific GP maps that was suggested to be highly evolvable is the modular GP map.

In order for complex organisms to be evolvable the GP map should be arranged in a way that avoids the constraining effects of pleiotropy. It was suggested that this is achieved by modularity, which means that functionally distinct character complexes are independently represented in the genome. In terms of pleiotropy this means that pleiotropic effects are

mainly limited to operate within character complexes and not between them (fig. 4) (Wagner and Altenberg 1996).
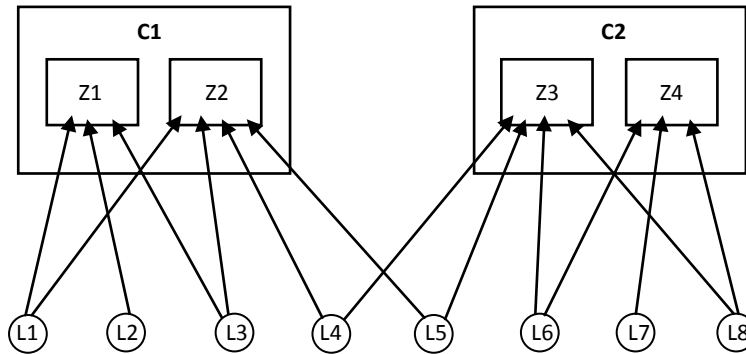

Figure 4 – Modularity: The effects (arrows) of the loci L1-L8 are mainly on characters (Z1-Z4) in the same character complex, C1 or C2. Only two of the loci (L4 and L5) affect parts of both character complexes. Modified from Wagner and Altenberg (1996).

Although pleiotropy is a common phenomenon, recent studies suggest that pleiotropy is in fact highly restricted and that the genotype-phenotype map is highly modular (Wagner and Zhang 2011).The results of a QTL study by Wagner et al. (2008a) of linear skeletal traits involving two inbred lines of mice were that the mean number of traits affected per gene was 7.8 (the median was 6), and there was a positive linear relationship between degree of pleiotropy (number of affected traits) and total mutational effect of a locus. They showed further that the average mutational effect per trait is increasing with the square root of the degree of pleiotropy (Wagner et al. 2008a). The "cost of complexity" is based on the assumption that all genes affect all traits, leading to the consequence of reduced probability of adaptive mutations as complexity increases (cf. Fisher's geometric model, section 2.2). It also assumes constant total effects, leading to a decrease of the average mutational effect per trait when the number of traits per gene increases. Both assumptions are refuted here, implying evolvability of complex organisms. However, a possible source of error in this study was the possibility that multiple mutations have been counted as one. That might have affected some of the conclusions, but does not revalidate the "cost of complexity" assumptions (Hermisson and McGregor 2008; Wagner et al. 2008b).

As pleiotropy has been regarded a constraint on evolution because it generates genetic covariances to which modularity might be the solution, this project is meant to contribute to

better understanding of how pleiotropy affects the response to selection, or the evolvability of phenotypes. I have investigated this question by simulation experiments, tracing the response across multiple generations given different genotype-phenotype maps (GP maps). In these individual-based simulations, the selection process is implemented via a fitness function, which assigns fitness to each individual in the population dependent on its trait values (the size of its traits).

2.4   The model

The GP map is represented by the B matrix model of Wagner (1989). The model is based on the assumption that the part of the genome underlying quantitative traits consists of two different types of genes, developmental genes and polygenes. Developmental genes are expressed in early development (typically during morphogenesis) and determine where different body parts are located and which genes are activated in which body parts. They can be for example homeotic genes. The quantitative variation of the different characters is however underlain by the polygenes, which can be any gene affecting the quantitative measures of the characters. In this way the pleiotropic structure is determined by the developmental genes, whereas the polygenes control quantitative measures of the characters but not the pleiotropy. The phenotype is represented by a vector $\boldsymbol{z}$ ($n \times 1$) containing the values of n traits. It is partitioned into a genetic ($\boldsymbol{x}$) and an environmental component ($\boldsymbol{e}$) (also $n \times 1$ vectors) such that:

$$\boldsymbol{z} = \boldsymbol{x} + \boldsymbol{e}, \tag{3}$$

where $\boldsymbol{e}$ is a random vector of mean zero and variance V_E. Each allele at the polygenic loci is represented by a value that quantifies its respective gene products' physiological property relevant for the genetic component $\boldsymbol{x}$. At each locus the value of the two alleles (assuming diploid organisms), which I will name the *allelic values*, are summed to generate the entries of the vector $\boldsymbol{y}$ of length equal to the number of polygenic loci L. The value of each entry of $\boldsymbol{y}$, the *y values*, is thus determined by the alleles at the respective locus, and represents a potential to affect the phenotype $\boldsymbol{z}$. Whether or not and to what degree it it will affect the different traits depends on the "developmental function", the GP map. This is represented by an $n \times L$ matrix $\boldsymbol{B}$ (the B matrix). The mapping is then done by linear transformation in the following way:

10

$$x = B \cdot y \tag{4}$$

This assumes additivity of allelic effects among loci. We further assume a constant B matrix (i.e. the developmental genes are not segregating), so the pleiotropic structure of the genome does not evolve. The phenotype is thus generated according to the following equation:

$$z = B \cdot y + e. \tag{5}$$

$$\Updownarrow$$

$$\begin{bmatrix} z_1 \\ z_2 \\ \vdots \\ z_n \end{bmatrix} = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & b_{1,L} \\ b_{2,1} & b_{2,2} & \dots & b_{2,L} \\ \vdots & \vdots & \ddots & \vdots \\ b_{n,1} & b_{n,2} & \dots & b_{n,L} \end{bmatrix} \cdot \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_L \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ \vdots \\ e_n \end{bmatrix}. \tag{5.1}$$

In my simulation studies I have considered the case with two characters ($n = 2$), thus equation (5) becomes:

$$\begin{bmatrix} z_1 \\ z_2 \end{bmatrix} = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & b_{1,L} \\ b_{2,1} & b_{2,2} & \dots & b_{2,L} \end{bmatrix} \cdot \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_L \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \tag{5.2}$$

Each column in the $2 \times L$-matrix represents a locus, and pleiotropy is defined independently for each locus as a non-zero entry in both rows (fig. 5).

$$\begin{bmatrix} 1 & 1 & \left(1\right. & 0 & 0 & 0 & 0 \\ 0 & 0 & \left.1\right) & 1 & 1 & 1 & 1 \end{bmatrix}$$

Figure 5 - Example of a B matrix with one pleiotropic locus (encircled).

In the case when the two entries of a column are of the same sign (as is the case of the encircled column in figure 5), the alleles at the respective locus will contribute to positive genetic covariance between the two traits, provided that the y value does not equal zero. I will refer to this as synergistic pleiotropy. If the two entries have opposite signs, that locus can contribute to negative covariance, so that will be referred to as antagonistic pleiotropy. Modularity in this context means that the two traits have independent genetic representations. A fully modular GP map is then represented by a B matrix with no pleiotropy (fig. 6).

Disregarding linkage disequilibrium, such a GP map will not generate genetic covariances, irrespective of the allelic values. The resulting G matrix takes the following form:

$$G = \begin{bmatrix} G_{1,1} & 0 \\ 0 & G_{2,2} \end{bmatrix}.$$

Another way such a G matrix can be generated, is if the pleiotropic effects are "hidden". This is the case when the GP map consists of both synergistic and antagonistic pleiotropy that cancel each other out (fig. 6). Thus, like with the modular GP map, no genetic covariance is generated.

$$\begin{bmatrix} 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \end{bmatrix} \text{(modular)}$$

$$\begin{bmatrix} 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & -1 & -1 & 0 & 0 & 0 & 0 \end{bmatrix} \text{(hidden pleiotropic)}$$

Figure 6 - Examples of B matrices which represent a modular (top) and a hidden pleiotropic (bottom) GP map.

3 Materials and Methods

3.1 Population simulations

The simulations were performed using R (http://www.r-project.org/, version 2.9.2). They involve the following steps. Consider a population of diploid organisms with L freely recombining loci underlying two traits. The population size (N) is fixed. It starts out with $2 \cdot N \cdot L$ unique alleles, which are mapped to the phenotype by the B matrix model. Assume further random mating, no mutation, no genotype-by-environment (GxE)-interactions, no epistatic effects on phenotype, no dominance effects and a constant B matrix structure. Individuals are selected according to a fitness function, with trait 1 being under positive directional selection and trait 2 under stabilizing selection. The process continues for t generations, and as there is no mutation, the genetic variation is reduced each generation, and the loci can eventually go to fixation. Statistical power is attained by doing c recursions. The main variables are shown below (table 1).

3.1.1 What the program does

A recursion loop starts by creating all the alleles in the initial population. To this end, $2L \cdot N$ allelic values ($\boldsymbol{y3}$) are drawn independently from a normal distribution with mean (m) and standard deviation (std). Before a generation loop is initiated, other necessary variables are defined: environmental component ($\boldsymbol{e}$), vector of summed allelic values ($\boldsymbol{y}$), genetic component ($\boldsymbol{x}$), optimal value of trait 2 ($^{opt}z_2$), fitness (W), mean population fitness ($\bar{W}$), relative fitness ($w = \frac{W}{\bar{W}}$).

To create the vector $\boldsymbol{y}$ of length L, for each individual, the two allelic values at each locus ($y3$) are summed. For the traits to start at positive values, a "start-vector" ($\boldsymbol{sv}$) is used to add the value 10 to both traits (eq. 6). The G matrix ($\boldsymbol{G}$) and a genetic correlation matrix $\boldsymbol{nG}$ are calculated based on the phenotype before environmental variation is included. A new vector of environmental variation components ($\boldsymbol{e}$), generated by drawing for each individual and each trait a number from a normal distribution with mean zero and chosen standard deviation ($estd$), is each generation added to the phenotype (rightmost addition in eq. 6), to complete

the trait values (z_1 and z_2). The optimal value of z_2 ($^{opt}z_2$) is set equal to the mean of trait 2 from the initial population without the environmental effects.

$$\begin{bmatrix} z_1 \\ z_2 \end{bmatrix} = \begin{bmatrix} sv_1 \\ sv_2 \end{bmatrix} + \begin{bmatrix} b_{1,1} & b_{1,2} & \ldots & b_{1,L} \\ b_{2,1} & b_{2,2} & \ldots & b_{2,L} \end{bmatrix} \cdot \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_L \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}. \qquad (6)$$

Table 1

Variables	
Name	Description
Input	
c	Recursions
t	Generations
N	Population size
s1	Selection coefficient of trait one
s2	Selection coefficient of trait two
m	Allelic mean
std	Allelic standard deviation
estd	Environmental standard deviation ($\sqrt{V_E}$)
B	B matrix
Output	
G[,,i,r]	G-matrix
nG[,,i,r]	Genetic correlation matrix
mG[,,i]	Mean G-matrix (across recursions)
nmG[,,i]	Mean genetic correlation matrix (across recursions)
XX1[r,i]	Population mean of trait one
XX2[r,i]	Population mean of trait two
X1[i]	Mean across recursions of population mean of trait one
X2[i]	Mean across recursions of population mean of trait two
gru[x]	Cumulative evolvabilities (the cumulative change in trait one per generation, from generation one to x, $x \in [2,G]$)
Other	
L	Number of loci
z1	Trait 1
z2	Trait 2
optz2	Optimum of trait 2
y3	Allelic values
x	Genetic component of phenotype
e	Environmental component of phenotype
W	Absolute fitness
w	Relative fitness

where *i* is a generation index, and *r* is a recursion index. The output variables are in matrix form, and the commas separate the different dimensions.

The fitness of each individual (W_j) is calculated according to the fitness function

$$W = 1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {}^{opt}z_2)^2, \tag{7}$$

and the individuals with negative fitness are counted. Negative fitness values are set to zero, corresponding to zero probability of reproducing. The population mean fitness ($\overline{W}$) and relative fitness of each individual ($w_j = \frac{W_j}{\overline{W}}$) are calculated. The relative fitness is used to generate a vector of individual-specific intervals, whose sizes correspond to the individuals' relative fitness. The intervals are scaled in order to cumulatively span a range [0,1], so that the individuals with the highest relative fitness are represented by the widest interval on the 0-1 span. The parents of every individual offspring being produced are then independently selected (with replacement) by drawing a number from a uniform distribution on the interval [0,1], the individuals with highest relative fitness having the highest probability of parenting the next generation. The selected individuals form a matrix with two columns of N individuals. The allelic values of both alleles at each locus of each selected individual are stored in a matrix.

The next step represents meiosis with freely recombining loci without crossing-over or mutation. A matrix is generated, consisting of two pairs of columns of zeros and ones, such that for each locus of each individual one homologous allele is represented by a zero and the other by a one with a 50% chance. The genetic composition of the offspring is generated by element-wise multiplication of this matrix with the matrix of allelic values, summed across homologous alleles for each parent, and with one column for each parent such that each offspring receives one allele from each parent per locus. The offspring allelic values are stored in $\mathbf{y3}$.

The population means of both traits are stored in each generation and recursion, resulting in the $c \times t$ matrices $\mathbf{XX1}$ and $\mathbf{XX2}$. This concludes the generation loop. A plot of the population mean trait values against the number of elapsed generations is shown after each recursion, and the recursion loop is ended.

Finally, the mean trait values across recursions are calculated from $\mathbf{XX1}$ and $\mathbf{XX2}$, and plotted against the number of elapsed generations (t). Other output variables are also calculated (table 1). The algorithm is also described in the flow diagram below (fig. 7).
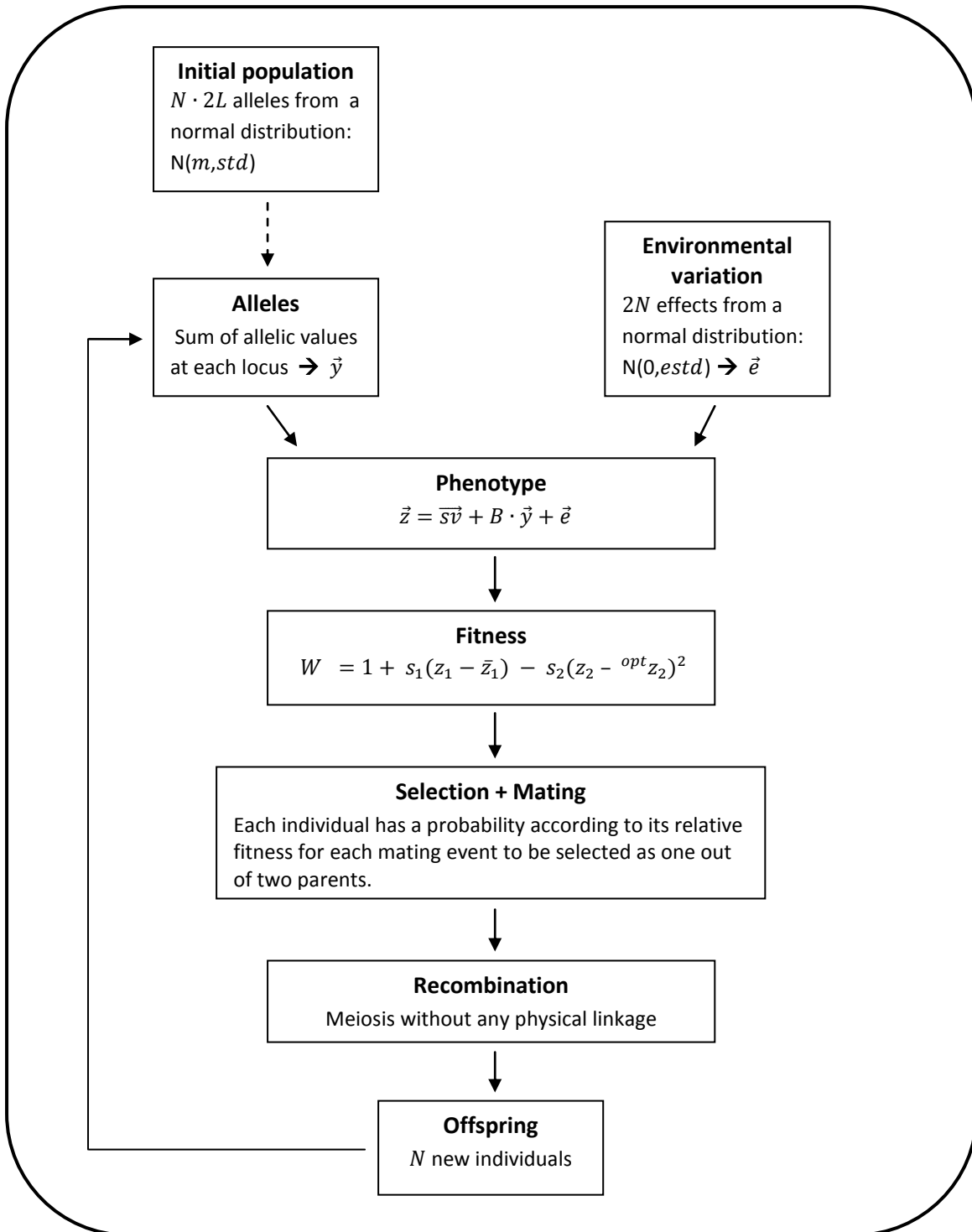
Figure 7 – Flow diagram for the algorithm

3.1.2 The choice of parameters

Negative fitness values are invalid and are set to zero in the course of the recursion. However, if all individuals have zero fitness, relative fitness values cannot be calculated. This situation is possible due to the fitness function (eq. 7): $W = 1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {}^{opt}z_2)^2$.

In the following I assume for simplicity that the initial allelic variance as well as the environmental variance in the population are equal to the corresponding variance of the distribution used to generate them. This is a good approximation as long as the population size (N) is sufficiently large, as the variance in the population (sample variance) is on average $\frac{N-1}{N}$ of the variance of the distribution used. In generation one (before selection) the alleles have expected value of zero and a variance std^2, according to the normal distribution they are drawn from ($N(0, std)$). Consider the case when the absolute values of all non-zero entries in the B matrix equal one and the B matrix has v such entries per trait. The expected value of z_1 and z_2 in generation one is then:

$E[z_k] = sv_k + E[B_k \cdot y] + E[e_k]$, $(e_k \sim N(0,1))$, where k is a trait index, making sv_k and e_k the start value and the environmental effect on trait k respectively, and B_k the k-th row of the B matrix. Thus $E[z_k] = sv_k = 10$, and the variance is:

$V[z_k] = V[B_k \cdot y] + V[e_k]$.

$V[z_k] = v \cdot 2 \cdot std^2 + 1$ (assuming independence among loci).

Since ${}^{opt}z_2$ is the mean value of z_2 (without environmental effects) in generation one $E[{}^{opt}z_2] = E[z_2]$, and ${}^{opt}z_2$ is an estimate of $E[z_2]$. Thus, $\frac{(z_2 - {}^{opt}z_2)^2}{V[z_2]}$ is approximately $\sim \chi_1^2$ and has $\mu = 1$ and $\sigma^2 = 2$ (because $\frac{(z_2 - {}^{opt}z_2)}{\sqrt{V[z_2]}} \sim N(0,1)$).

$\Rightarrow E[(z_2 - {}^{opt}z_2)^2] = V[z_2] \cdot 1$ and $V[(z_2 - {}^{opt}z_2)^2] = V[z_2]^2 \cdot 2$.

Then $E[W]$ becomes: $1 + s_1 \cdot E[z_1 - \bar{z}_1] - s_2 \cdot E[(z_2 - {}^{opt}z_2)^2]$.

$E[W] = 1 - s_2 \cdot V[z_2] = 1 - s_2 \cdot (n \cdot 2 \cdot std^2 + 1)$.

And $V[W]$ becomes $s_1^2 \cdot V[z_1 - \bar{z}_1] + s_2^2 \cdot V[(z_2 - {}^{opt}z_2)^2]$ (assuming independent traits).

$$V[W] = s_1^2 \cdot V[z_1] + s_2^2 \cdot 2V[z_2]^2.$$

$$V[W] = s_1^2 \cdot (n \cdot 2 \cdot std^2 + 1) + s_2^2 \cdot 2(n \cdot 2 \cdot std^2 + 1)^2.$$

$$V[W] = s_1^2(n \cdot 2 \cdot std^2 + 1) + 2s_2^2(n \cdot 2 \cdot std^2 + 1)^2.$$

In any generation $E[W]$ is

$$E[W] = E\left[1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {}^{opt}z_2)^2\right] = 1 - s_2 \cdot E\left[(z_2 - {}^{opt}z_2)^2\right].$$

This equals

$$1 - s_2 \cdot E\left[z_2^2 - 2^{opt}z_2 \cdot z_2 + {}^{opt}z_2{}^2\right] = 1 - s_2 \cdot \left(E[z_2^2] - 2^{opt}z_2 \cdot E[z_2] + {}^{opt}z_2{}^2\right)$$

$$= 1 - s_2 \cdot \left(V[z_2] + E[z_2]^2 - 2^{opt}z_2 \cdot E[z_2] + {}^{opt}z_2{}^2\right).$$

$$\Rightarrow E[W] = 1 - s_2 \cdot (V[z_2] + (E[z_2] - {}^{opt}z_2)^2). \tag{8}$$

It is reasonable to set the parameter values such that $E[W] > 0$. If a large portion of the population is wiped out due to negative fitness values this can greatly reduce the effective population size and thus affect the evolutionary dynamics.

For convenience I set the environmental variance equal to one and use this as a reference value when setting the other parameters. The trait values will then be given as units of environmental variance (V_E).

In order for the population to have a realistic level of heritability (h^2) and $V_E = 1$, attention must be paid to what levels of additive genetic variance (V_A) can be used.

The heritability $h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_A + V_E}$, $z = B \cdot y + e$, $z_k = B_k \cdot y + e_k = \sum_{l=1}^{L} b_{k,l} \cdot y_l + e_k$, where l is a locus index. The term $e_k \sim N(0, estd^2) = N(0,1) \Rightarrow V_E = 1$. $y_l = y3_{1,l} + y3_{2,l}$, $y3_{p,l} \sim N(m, std^2)$, where p is a gamete index and all $y3_{p,l}$ are independent $\Rightarrow Var(y_l) = 2std^2$. The terms $y3_{p,l}$ and e_k are independent $\Rightarrow Var(z_k) = 2std^2 \sum_{l=1}^{L} b_{k,l}^2 + 1 = V_P$, where

$$V_A = 2std^2 \sum_{l=1}^{L} b_{k,l}{}^2 \tag{9}$$

18

and $V_E = 1$, $=> h^2 = \frac{2std^2 \sum_{l=1}^{L} b_{k,l}^2}{2std^2 \sum_{l=1}^{L} b_{k,l}^2 + 1}$. For example, setting h^2 equal to 0.50 implies that

$std^2 \sum_{l=1}^{L} b_{k,l}^2 = \frac{1}{2}$. In the first experiment, I set $\sum_{l=1}^{L} b_{k,l}^2 = 10$. In order to get h^2 equal to 0.50, std^2 must be equal to 0.05, thus std must equal $\sqrt{0.05} \approx 0.224$. Notice that this is the initial heritability before genetic variation is lost by selection and drift. In the experiments the std I used was 0.5 making the initial $h^2 = \frac{2 \cdot 0.5^2 \cdot 10}{2 \cdot 0.5^2 \cdot 10 + 1} = \frac{5}{6} \approx 0.833$. This is a reasonable value.

3.1.3   The fitness function

The fitness function (eq. 7) combines directional selection on trait 1 (z_1) with stabilizing selection on trait 2 (z_2). Trait 1 is the focal trait whose response to selection may be constrained when it is correlated with trait 2, as selection tries to maintain the latter at an optimal value ($^{opt}z_2$). The coefficients of selection, s_1 and s_2, determine the strength of selection on trait 1 and trait 2 respectively. Stabilizing selection is achieved by a quadratic function ($-s_2(z_2 - {^{opt}z_2})^2$) such that fitness is reduced proportionally to the squared deviation from the optimum in any direction. The strengths of directional selection are expressed in terms of the selection gradient ($\boldsymbol{\beta}$), $\beta_1 = \frac{\partial w}{\partial z_1}$, $\beta_2 = \frac{\partial w}{\partial z_2}$. Relative fitness equals individual absolute fitness divided by mean population fitness, $w = \frac{W}{\overline{W}}$. Thus according to the fitness function, $W = 1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {^{opt}z_2})^2$,

$$\beta_1 = \frac{s_1}{\overline{W}}, \tag{10}$$

where $\overline{W} = E[W] = 1 - s_2 \cdot (V[z_2] + (E[z_2] - {^{opt}z_2})^2)$ (cf. eq. 8). If we consider the case with only directional selection on trait 1 and no stabilizing selection on trait 2 ($s_2 = 0$), then $\overline{W} = 1$ and $\beta_1 = s_1$. Further, the strength of selection on trait 2, which occurs when the trait is displaced from its optimum, can be estimated as follows:

$$\beta_2 = \frac{\partial w}{\partial z_2} \implies \beta_2 = \frac{-2s_2(z_2 - {^{opt}z_2})}{\overline{W}} \approx \frac{-2s_2(E[z_2] - {^{opt}z_2})}{\overline{W}}. \tag{11}$$

3.1.4   Selection response

When measuring the response to selection, I contrast two different measures, the *selection limit* and the *short-term response*. The selection limit is defined as the total evolutionary

change achievable. In practice this value is calculated by subtracting the mean phenotypic value in the first generation from the mean value in some distant generation where I assume no more change will happen. This assumption is based on the observation that the mean phenotype has not changed significantly for several generations, and that only a minimal amount of additive genetic variance underlying the focal trait remains. The short-term response is measured either as a cumulative response over some time interval, divided by the number of generations passed in that interval, or simply as the total change in mean phenotype from the first generation to some later generation. The point here is however that the level of underlying additive genetic variance is substantial throughout the time span. The numbers are generally mean values over c recursions. Confidence intervals (CI) have 95% confidence level.

3.2 Experiments

3.2.1 Hidden pleiotropy

The first problem I treated was whether hidden pleiotropy constrains evolution. In this experiment I have compared the selection response over many generations given different GP maps. The GP maps all have the same expected amount of initial additive genetic variance underlying trait 1. I first considered the question whether hidden pleiotropy reduces the evolvability of trait 1, compared to a modular GP map structure with the same genetic effects on the focal trait 1.

Parameter settings: $c = 20$, $t = 751$, $N = 200$, $L = 20$, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0.5$.

Initially $V_A = 2std^2 \sum_{j=1}^{L} b_{k,l}^2$ (eq. 9) and $\sum_{j=1}^{L} b_{k,l}^2 = 10$

$=> V_A = 5$.

The response to selection of trait 1 is measured as the difference between the initial population mean and the mean after 750 (t-1) generations of selection. The values are given as the mean over c recursions. The program is run seven times, with seven different B matrices (B1-B7, listed below). The B matrices include one with all effects being synergistically pleiotropic (fully constrained), one with no pleiotropy (fully modular), one with all effects being pleiotropic, but with equal amounts of synergistic and antagonistic pleiotropy (fully

20

hidden pleiotropy), and four others with *partially hidden pleiotropy*. By partially hidden pleiotropy I mean that there is both synergistic and antagonistic pleiotropy present, such that they partially but not completely cancel each other out (see B matrices below).

B-matrices:

$$B1 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(fully constrained)

$$B2 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(20% hidden)

$$B3 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(40% hidden)

$$B4 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(60% hidden)

$$B5 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(80% hidden)

$$B6 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & -1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

(fully hidden pleiotropy)

$$B7 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

(fully modular)

$$B8 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

(no effects on trait 2).

The expected initial G matrix in the cases of both the modular and the fully hidden pleiotropic GP map equals:

$$G = \begin{bmatrix} 5 & 0 \\ 0 & 5 \end{bmatrix}.$$

According to the Lande equation (eq. 1 and 2) the response should also be the same:

$$\Delta \bar{z}_1 = G_{1,1} \, \beta_1 + G_{1,2}\beta_2 = 5\beta_1.$$

However, as this is only the expected *initial* G matrix, when evolution changes allele frequencies and by chance fixes alleles with not exactly the same allelic values at the different loci, covariances can appear in the case of hidden pleiotropy and the term $G_{1,2}\beta_2$ may deviate from zero. The modular GP map, however, avoids genetic covariances irrespective of allele frequencies. Thus, my hypothesis is that trait 1 will show a lower response when underlain by a 100% pleiotropic GP map (B matrix 6), than when underlain by a fully modular map (B matrix 7).

3.2.2   Mouse B matrix

Further, an approximate real B matrix was tested for comparison. This B matrix was provided by Mihaela Pavlicev, and based on estimated effects on different quantitative traits from 546 QTLs from the F2/F3 generations of mouse intercross between large (LG/J) and small (SM/J) inbred lines of the Cheverud lab (for details on the intercross see Cheverud et al. (1996)). The two traits I used were femur and humerus length. In order for this 546-loci B matrix to be comparable to the above B matrices, I grouped loci with similar pleiotropy together in "super loci" and scaled the effect sizes to give the same initial additive genetic variance as the others. The effects of a locus (or rather the potential effects in the case of the B matrix entries $b_{1,l}$ and $b_{2,l}$) can be regarded as a vector $\boldsymbol{b_l}$ in the two dimensional phenotype space in the two-trait scenario (fig. 8). As the above pleiotropic B matrices have 10 non-zero loci, I have constructed 10 "super loci" by partitioning the circle representing the possible directions of $\boldsymbol{b_l}$ in 10 equally sized sectors, and summed all loci that fall into each sector according to their direction in phenotype space. This resulted in the following B matrix:

$$\begin{bmatrix} 1.494537 & 2.769312 & 0.0007735036 & -0.04667998 & -0.006092008 & -0.02388131 & -0.2719293 & 0 & 0 & 0.1433124 \\ 0.764224 & 2.998482 & 0.3087174 & 0.07636481 & 0.001893725 & -0.01275512 & -0.2862922 & 0 & 0 & -0.0513877 \end{bmatrix}$$
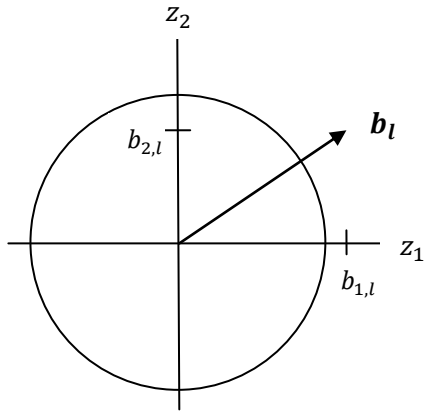
Figure 8 – The locus b_l is a vector $[b_{1,l}, b_{2,l}]$ in the phenotype space.

It was generated in this way in order for it to be able to represent all possible directions in phenotype space. Note, however, that the distribution of the b_l here is highly skewed (most of the effects are at the two first loci). I used the same parameter settings as in section 3.2.1 for the simulations.

3.2.3   The effect of number of loci

An experiment was performed to test whether there is a difference in selection response of trait 1 (conditional on stabilizing selection on trait 2), and in how the G matrix changes, between a scenario with few loci with large effects and a scenario with many loci with small effects, provided equal expected initial G matrix.

Parameter settings: $c = 20$, $t = 751$, $N = 200$, $L = 2$ or 50, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0.5$.

Initially (before selection) $V_A = 2std^2 \sum_{l=1}^{L} b_{k,l}^2$ (eq. 9), where k is the trait index and l the locus index, thus making $b_{k,l}$ the entry of row k and column l in $\mathbf{B}$.

$$\sum_{l=1}^{L} b_{k,l}^2 = 10.$$

I used $std = 0.5$ for both GP maps, and varied the values of the $b_{1,l}$'s. Both GP maps were modular with no effects on trait 2 ($b_{2,l} = 0$).

(a) With two loci of equal potential effects ($b_{k,l}$) on trait 1:

$$5 = 2 \cdot 0.5^2 \cdot 2b_{1,l}^2 \quad => \quad b_{1,l} = \sqrt{5} \approx 2.236068.$$

(b) With 50 loci of equal potential effects ($b_{k,l}$) on trait 1:

$$5 = 2 \cdot 0.5^2 \cdot 50 b_{1,l}{}^2 \quad => \quad b_{1,l} = \frac{1}{\sqrt{5}} \approx 0.4472136.$$

B matrices:

$$\begin{bmatrix} 2.236068 & 2.236068 \\ 0 & 0 \end{bmatrix} (L = 2)$$

$$\begin{bmatrix} 0.4472136 & 0.4472136 & \dots & 0.4472136 \\ 0 & 0 & \dots & 0 \end{bmatrix} (L = 50).$$

In both cases the expected initial G matrix is thus:

$$G = \begin{bmatrix} 5 & 0 \\ 0 & 0 \end{bmatrix}.$$

3.2.4   Alternative allelic distributions

Theory suggests that the question of whether hidden pleiotropy constitutes a constraint depends highly on the distribution of allelic effects in the population (Wagner 1989; Slatkin and Frank 1990). It is expected to do so when the distribution is not normal, and not to do so when it is. In my model system the allelic values are drawn from a normal distribution and the effects are expected to be normally distributed, at least initially. The simulation studies of Baatz and Wagner (1997) and Griswold (2006) found that hidden pleiotropy slows down the evolution of a trait under directional selection when it is pleiotropically connected to one under stabilizing selection. However, the model systems they used do not assume normally distributed allelic effects. To address the question of whether it is the distribution of allelic effects that determines the impact of hidden pleiotropy on evolution, I have adjusted my model system to conform to the studies of Baatz and Wagner (1997) and Griswold (2006) (next section).

The Baatz and Wagner (1997) study uses a model where one trait is under directional selection and another trait is under stabilizing selection. The authors used the following fitness function: $W(\mathbf{z}) = exp\left(\sigma z_1 - \frac{z_2^2}{2\omega_2^2} \right)$, and they included both a deterministic and a stochastic model. The deterministic model (two-locus, two-allele) can be represented by the B matrix model as follows: $B = \begin{bmatrix} d & d \\ d & -d \end{bmatrix}$, where the allelic values are either 0 or 1. They showed that the rate of evolution of the focal character is reduced by a term proportional to

$Cov(x_1, x_2^2)$ (where $z = x + e$), and they find that when the initial frequency of the alleles with value 1 (q) is low then the evolution of trait 1 is inhibited, whereas when the initial frequency of those alleles is high the evolution of trait 1 is accelerated. This is because of the effects the allele frequencies have on the $Cov(x_1, x_2^2)$ term. When the allele of value 1 is rare, high values of trait 1 are associated with more extreme values of trait 2 (or x_2^2) because of the asymmetry resulting from the hidden pleiotropy structure of the GP map. When the allele of value 0 is rare, low values of trait 1 are associated with more extreme values of trait 2 and the term $Cov(x_1, x_2^2)$ becomes negative, thus accelerating the evolution of trait 1. This means that the evolutionary change in trait 1 is reduced by pleiotropy when it is associated with an increase in the variance of trait 2, and enhanced when the change in trait 1 is associated with a decrease in the variance of trait 2. It makes biologically sense that when the hidden pleiotropy structure gives an additional selective pressure through trait 2 on the alleles either in accordance with or in opposite direction of the selective pressure induced by the directional selection alone, this can promote or impede the evolution of trait 1, respectively.

In my experiments the alleles are drawn from a normal distribution, and I use a much higher heritability than Baatz and Wagner (1997), so the dynamics could easily be somewhat different. I also use generally weaker stabilizing selection and this could as well have an effect. Normally distributed allelic effects enable the increase in fitness by substitution at one locus at a time, because for some small value of change at a locus affecting both traits, if trait 2 is close enough to its optimum, fitness can be increased. It is the relative sizes of s_1 and s_2 and the distance of trait 2 from its optimum that determines how small this value must be. It is natural to repeat some of the simulations with some approximated s_1 and s_2 values:

$$W = exp\left(\sigma z_1 - \frac{z_2^2}{2\omega_2^2}\right) => \frac{\partial W}{\partial z_1} = \sigma \cdot exp\left(\sigma z_1 - \frac{z_2^2}{2\omega_2^2}\right). \text{ Since } \beta_k = \frac{\partial W}{\partial z_k}\Big/\overline{W}, \text{ approximating } W$$

with $\overline{W}$ gives $\beta_1 \approx \sigma$ and $\beta_2 \approx -\frac{z_2}{\omega_2^2}$, which can be approximated as $-\frac{\overline{z_2}}{\omega_2^2}$. In terms of the fitness function used in my experiments $W = 1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {}^{opt}z_2)^2$ if we assume $\overline{W} \approx 1$ and ${}^{opt}z_2 \approx 0$, then $s_1 \approx \sigma$ and $s_2 \approx \frac{1}{2\omega_2^2}$. Values comparable to those used by Baatz and Wagner are thus e.g. $s_1 = 0.2$ and $s_2 = 0.3$.

To compare between the Baatz and Wagner (1997) setting and my own I simulated the response to selection with a hidden pleiotropic GP map and a modular one once in a two-

allele setting similar to the one used by Baatz and Wagner (1997), and in a setting with normally distributed alleles, as I have generally been using. In both settings I have used "my" fitness function (eq. 7) with the values derived above, and B matrices with only two loci.

For the two-allele setting a new parameter was introduced, namely the initial frequency of the allele with value 1 (q). The other allele has value 0 and initial frequency 1-q.

Parameter settings: $c = 20$, $t = 151$, $N = 1000$, $L = 2$, $s_1 = 0.2$, $s_2 = 0.3$, $q = 0.02$ (biallelic setting), $std = 0.14$ (normal setting), $V_E = 1$.

B matrices:

$$B = \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix} \text{ (hidden pleiotropic)}$$

$$B = \begin{bmatrix} 1 & 1 \\ 0 & 0 \end{bmatrix} \text{ (modular)}.$$

Expected initial G matrices:

$$G = \begin{bmatrix} 0.0784 & 0 \\ 0 & 0.0784 \end{bmatrix} \text{ (both settings, pleiotropic B matrix)}.$$

$$G = \begin{bmatrix} 0.0784 & 0 \\ 0 & 0 \end{bmatrix} \text{ (both settings, modular B matrix)}.$$

In this experiment the modular matrix has only zero effects on trait 2 and thus also avoids the potential constraining effects of linkage disequilibrium. I have used a larger population size here than in the other simulations to cope with the stochasticity resulting from the much lower heritability (initial value: $h^2 = \frac{V_A}{V_A + V_E} = 0.073$).

3.2.5   Mutation-based simulations

The simulations of Baatz and Wagner (1997) and Griswold (2006) finding constraining effects of hidden pleiotropy involved mutation. In order to do a comparable analysis, the relevant experiments should be repeated with the inclusion of mutation. I thus modified my program to add at random a mutational value from a normal distribution, $N(0, mstd)$, to a fraction u of the alleles after each generation of selection. The population was initially

genetically homogenous, and the following parameter values were used: $c = 20$, $t = 1001$, $N = 200$, $L = 10$, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0$, $mstd = 0.5$, $u = 0.001$.

B matrices:

$$B = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \text{ (modular without constraining LD effects)},$$

$$B = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & -1 & -1 & -1 & -1 & -1 \end{bmatrix} \text{ (100\% hidden pleiotropic)}.$$

Both are expected to have the same initial V_A underlying trait 1, and no initial genetic covariances. When it comes to the mutation rate (u), I have used a number that would correspond to $\dfrac{u \cdot N \cdot L \cdot 2 \text{ mutations per generation}}{2 \text{ gametes per new individual } \cdot N \text{ individuals}} = u \cdot L = 0.01$ per gamete per individual. The corresponding rate in Griswold (2006) was 0.001.

3.2.6   Even B matrix

If directional selection is assumed not to be limited to the direction of trait 1 in phenotype space, but rather to point in any arbitrary direction $\boldsymbol{\beta}$ with stabilizing selection in all other directions, Hansen (2003) predicts maximum evolvability for a GP map that is fully pleiotropic but with maximally variable pleiotropic effects. To test this hypothesis, I let the selective regime (and the other parameter values) remain as in the previous cases (section 3.2.1) and construct a B matrix with maximally different pleiotropic loci.

It is, however, not obvious that the exact directions of these loci of limited number are immaterial with respect to evolvability. I have created an *even B matrix* (fig. 9 a):

$$\begin{bmatrix} 1.414214 & 1.144123 & 0.437016 & -0.437016 & -1.144123 & -1.414214 & -1.144123 & -0.437016 & 0.437016 & 1.144123 \\ 0 & 0.8312539 & 1.344997 & 1.344997 & 0.8312539 & 1.731855e-16 & -0.8312539 & -1.344997 & -1.344997 & -0.8312539 \end{bmatrix}$$

Interchanging the rows of it generated another *reversed even B matrix* (fig. 9 b):

$$\begin{bmatrix} 0 & 0.8312539 & 1.344997 & 1.344997 & 0.8312539 & 1.731855e-16 & -0.8312539 & -1.344997 & -1.344997 & -0.8312539 \\ 1.414214 & 1.144123 & 0.437016 & -0.437016 & -1.144123 & -1.414214 & -1.144123 & -0.437016 & 0.437016 & 1.144123 \end{bmatrix}$$
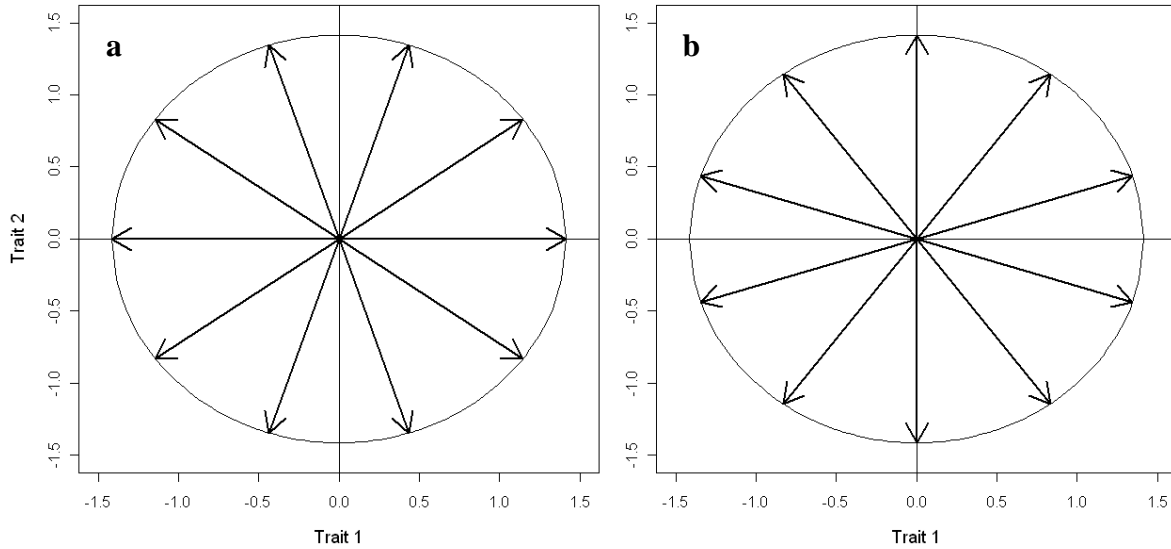
Figure 9 – Graphic representation of the even (a) and the reversed even (b) B matrix, the arrows representing the different loci in terms of their *potential effects* b_l, which are all of equal length ($\sqrt{2}$).

At least for the selection limit, I predict that even though both matrices yield the same expected initial G matrix, $G = \begin{bmatrix} 5 & 0 \\ 0 & 5 \end{bmatrix}$, the one with the highest sum of absolute values of the entries of B mapping to trait 1 ($b_{1,l}$) would have higher evolvability (selection limit) according to the rationale in section 4.1.4 (If we assume fixation of a maximum allelic value d at all loci, it is the absolute values at the loci affecting trait 1 ($b_{1,l}$) that determine total evolutionary response, not the squared values which underlie the G matrix). This is the even B matrix, for which this number equals 9.152982, whereas for the reversed even B matrix, this sum equals 8.705004. The corresponding number for both the 100% hidden pleiotropic and the modular GP map (section 3.2.1), which also yield the above mentioned G matrix, is 10. I thus predict the even B matrices to have slightly lower responses then the hidden pleiotropic and the modular.

3.2.7   Partial hidden pleiotropy vs. partial modularity

Gromko (1995) investigated the variability of correlated responses using computer simulations. He showed that different pleiotropic structures of the GP map with the same genetic correlations can yield different levels of variation in correlated responses among selection lines. He compared GP maps with some loci generating correlations between two

28

traits and some loci being either pleiotropic or modular. Two of the GP maps that were compared can be represented by the following B matrices:

$$B = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & -1 & -1 \end{bmatrix} \quad \text{(partially hidden pleiotropic)}$$

and

$$B = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \end{bmatrix} \quad \text{(partially modular)},$$

corresponding to Gromko's pleiotropy structures (6,2,0) and (4,0,8) respectively. These GP maps are interesting for my study because although they result in the same expected initial G matrix, my hypothesis here is that the modular GP map would result in a significantly higher evolvability than the hidden pleiotropic one, because the modular GP map has unconstrained loci affecting trait 2 that could completely compensate for the correlated responses generated by the pleiotropic loci. The hidden pleiotropic system does not have this possibility. An experiment was done to test this hypothesis. The following parameter values were applied: $c = 20$, $t = 351$, $N = 200$, $L = 8$ or 12, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0.5$. I used the two above-mentioned B matrices. Expected initial G matrix for both configurations:

$$G = \begin{bmatrix} 4 & 2 \\ 2 & 4 \end{bmatrix} \text{ (i.e. the genetic correlation is 50\%)}.$$

3.2.8   Linkage disequilibrium

In addition I tested the validity of the assumption that linkage disequilibrium is an unimportant factor in my experiments. Because covariance due to linkage disequilibrium is the only constraining factor acting in the case of a modular GP map, I compared the evolution of trait 1 when using B7 with the evolution of trait 1 when using a B matrix with no effects on trait 2 (B8). In that case LD cannot generate any genetic covariances between trait 1 and trait 2 as there is no additive genetic variance underlying the latter. I used the same parameter settings as in section 3.2.1. The expected initial G matrix is thus:

$$G = \begin{bmatrix} 5 & 0 \\ 0 & 0 \end{bmatrix}.$$

3.2.9   Effects of the strength of stabilizing selection

In this experiment, I compared the evolutionary responses of hidden pleiotropic and modular GP maps with varying levels of stabilizing selection (s_2) in order to check the robustness of the results from the experiments in section 3.2.1.

Parameter settings: $c = 20$, $t = 751$, $N = 200$, $L = 20$, $s_1 = 0.1$, s_2 = variable, $std = 0.5$.

B matrices: B6, B7 and B1 (shown above).

3.2.10   Pleiotropy as a source of variation

Can pleiotropy increase evolvability by acting as a source of variation? In this experiment I have investigated how pleiotropy can increase evolvability by letting more loci affect the focal trait. This rests on different assumptions than the previous experiments. There the total variation underlying the focal trait was fixed by always having the same top row of the B matrix in terms of $V_A = 2std^2 \sum_{j=1}^{L} b_{k,l}^2$ (eq. 9). Then by increasing the level of pleiotropy, more and more of these L loci would also affect the trait under stabilizing selection, and the focal trait could only be more constrained. This was a useful approach for comparing different levels of hidden pleiotropy and modularity. However, with a fixed number of loci underlying all quantitative traits in the genome, the more pleiotropic the loci are on average, the more loci will on average affect each trait. If the total effect of a locus is not constant but increases with the number of affected traits, then the total genetic variation underlying each trait should on average increase with increasing average pleiotropy. According to Wagner, Kenney-Hunt et al. (2008a) this seems indeed to be the case, as an approximately linear relationship between total effect size and number of traits affected was observed. A question that arises when the possibility of this positive effect of pleiotropy on evolvability is considered is, what the optimal level of pleiotropy would be, as the constraining effects of correlated responses still would be present. This was investigated in the following way.

I again compared the evolutionary response of the focal trait between different GP maps. However, I used 350 generations of selection and 50 loci. All entries in the B matrix in this experiment were either equal to 1 or 0. I started with a completely modular map, with 25 different loci affecting each trait. Then I used a B matrix where one of the loci only affecting trait 1 and one of the loci only affecting trait 2 were changed to also have an equal effect on

the other trait, thus being (2 out of 50 loci being pleiotropic) 4% pleiotropic. I did this for all levels of pleiotropy (0%, 4%, 8%, 12%, 16%,…, 100%) and compared the response to selection on trait 1.

Parameter settings: $c = 20$, $t = 351$, $N = 200$, $L = 50$, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0.5$.

Thus, the initial $V_A = 2std^2 \sum_{j=1}^{L} b_{k,l}^2$ increases with increasing pleiotropy.

B-matrices:

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 25)$$

(0% pleiotropy)

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & 0 & 0 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 26)$$

(4% pleiotropy)

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & 0 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 27)$$

(8% pleiotropy)

…

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \\ 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 50)$$

(100% pleiotropy).

The expected initial G matrices are thus:

$$G = \begin{bmatrix} 12.5 & 0 \\ 0 & 12.5 \end{bmatrix} \quad \text{(0% pleiotropy),}$$

$$G = \begin{bmatrix} 13 & 1 \\ 1 & 13 \end{bmatrix} \quad \text{(4% pleiotropy),}$$

$$G = \begin{bmatrix} 13.5 & 2 \\ 2 & 13.5 \end{bmatrix} \quad \text{(8% pleiotropy),}$$

…

$$G = \begin{bmatrix} 25 & 25 \\ 25 & 25 \end{bmatrix} \quad \text{(100\% pleiotropy)}.$$

3.2.11   Predictive value of the conditional evolvability

When a trait under directional selection (trait 1) is genetically connected to another trait under stabilizing selection (trait 2) Hansen (2003) has shown that the evolvability of trait 1 is determined by its *conditional genetic variance*, assuming multivariate normally distributed allelic effects. This variance predicts the evolutionary response of trait 1 after equilibrium between the different selective forces working on the two traits has been obtained, such that the initial displacement of trait 2 caused by correlated response has reached a certain value and stopped responding. The response in trait 1 is then estimated by multiplying the conditional additive genetic variance of trait 1 with its selection gradient (β_1). The conditional genetic variance ($G_{Z_1|Z_2}$) of trait 1 on trait 2 equals the additive genetic variance of trait 1 minus the squared additive genetic covariance between trait 1 and trait 2 divided by the additive genetic variance of trait 2, or the additive genetic variance of trait1 times the fraction of it being independent of potential constraining characters (its *autonomy*) (Hansen and Houle 2008). In terms of the G matrix: $G_{Z_1|Z_2} = G_{1,1} - \frac{G_{1,2}^2}{G_{2,2}}$. Thus,

$$\Delta \bar{z}_1 = G_{Z_1|Z_2} \cdot \beta_1 = \left(G_{1,1} - \frac{G_{1,2}^2}{G_{2,2}} \right) \cdot \frac{s_1}{\bar{w}}.$$

I have tested the predictive value of this *conditional evolvability* and compared it with the univariate version of the Lande's equation ($\Delta \bar{z}_1 = G_{1,1} \cdot \beta_1$) as well as the bivariate (i.e. complete in the case of two traits) Lande equation ($\Delta \bar{z}_1 = G_{1,1} \cdot \beta_1 + G_{1,2} \cdot \beta_2$). The parameter β_2 is approximated in the following way:

$$\beta_2 = \frac{-2s_2(z_2 - {}^{opt}z_2)}{\bar{W}} \approx \frac{-2s_2(E[z_2] - {}^{opt}z_2)}{\bar{W}}.$$

As the Lande equation assumes multivariate normal distribution of allelic effects, and the conditional evolvability uses the same equation with a constrained variance, it is not evident that either will predict well, since the allelic distribution changes through the course of evolution.

Parameter settings: $c = 20$, $t = 201$, $N = 200$, $L = 50$, $s_1 = 0.1$, $s_2 = 0.02$, $std = 0.5$.

B matrices:

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 34)$$

(36% pleiotropy, only synergistic)

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 42)$$

(68% pleiotropy, only synergistic)

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & -1 & -1 & -1 & -1 & -1 & -1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 34)$$

(36% pleiotropy, only antagonistic)

$$B = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & -1 & -1 & -1 & -1 & -1 & -1 & \cdots & 1 & 1 & 1 \end{bmatrix} \quad (\sum_{j=1}^{L} b_{k,l}^2 = 42)$$

(68% pleiotropy, only antagonistic).

4 Results

4.1.1 Hidden pleiotropy

Here I investigated whether a hidden pleiotropic GP map reduced the evolvability of trait 1 compared to a modular map. Examples of the simulation procedure are shown in figure 10. In figure 10a and b, a modular B matrix is used and trait 2 is genetically independent from trait 1, and it is kept at the same value by stabilizing selection. Trait 1, being subject to positive directional selection, has a clear response the first few generations while there is a considerable amount of additive genetic variance underlying it. After about 200 generations, the genetic variation is depleted and the trait stops responding.

In figure 10c and d, the GP map is pleiotropic and a lower response is demonstrated. All loci with non-zero effects are pleiotropic, with 80% of them being synergistic and 20% of them being antagonistic such that 40% of the pleiotropy is hidden (B3). After about 100 generations trait 1 stops responding although it still has some amount of additive genetic variance. This variation is, however, not independent from the additive genetic variance of trait 2, as can be seen from the correlation graph. Thus according to the Lande equation, the stabilizing selection on trait 2 will through covariances induce a negative selection pressure on trait 1 diminishing its response: $\Delta \bar{z}_1 = G_{1,1} \beta_1 + G_{1,2} \beta_2$. The term $\Delta \bar{z}_1$ is reduced whenever $G_{1,2}$ is non-zero and has an opposite sign to β_2. In this example $G_{1,2}$ is positive (cf. fig. 10d) and β_2 is negative whenever trait 2 is larger than the optimum (whose expected value is 10). ($G_{1,1}$ is the additive genetic variance of trait 1 and $G_{1,2}$ is the additive genetic covariance between trait 1 and trait 2.) This effect goes the opposite way as well, as trait 2 is slightly increased. In this way pleiotropy is constraining the adaptive evolution of trait 1.

The selection coefficients (s_1 and s_2) were set such that a huge difference in response in trait 1 between the scenario with the fully constrained GP map (B1) and the on with the modular map (B7) would be observed. The results are given as the mean output values from 20 recursions. The response values in these two cases were 2.42 (constrained) and 23.14 (modular). The values are in units of environmental variance with expected initial heritability equal to $\frac{5}{6}$. In the constrained case a fast response is seen the first few generations, that very quickly halts and remains at the same level for the rest of the evolutionary time (fig. 11a). As

figure 11a shows, mean values of the two traits are very similar, as are their underlying genetic variances (fig. 11b). The two traits are genetically completely correlated, as can be seen from the correlation graph. As in the second example above (fig. 10), the response stops although there still is a certain level of additive genetic variance, implying that conflicting selection pressures are present.
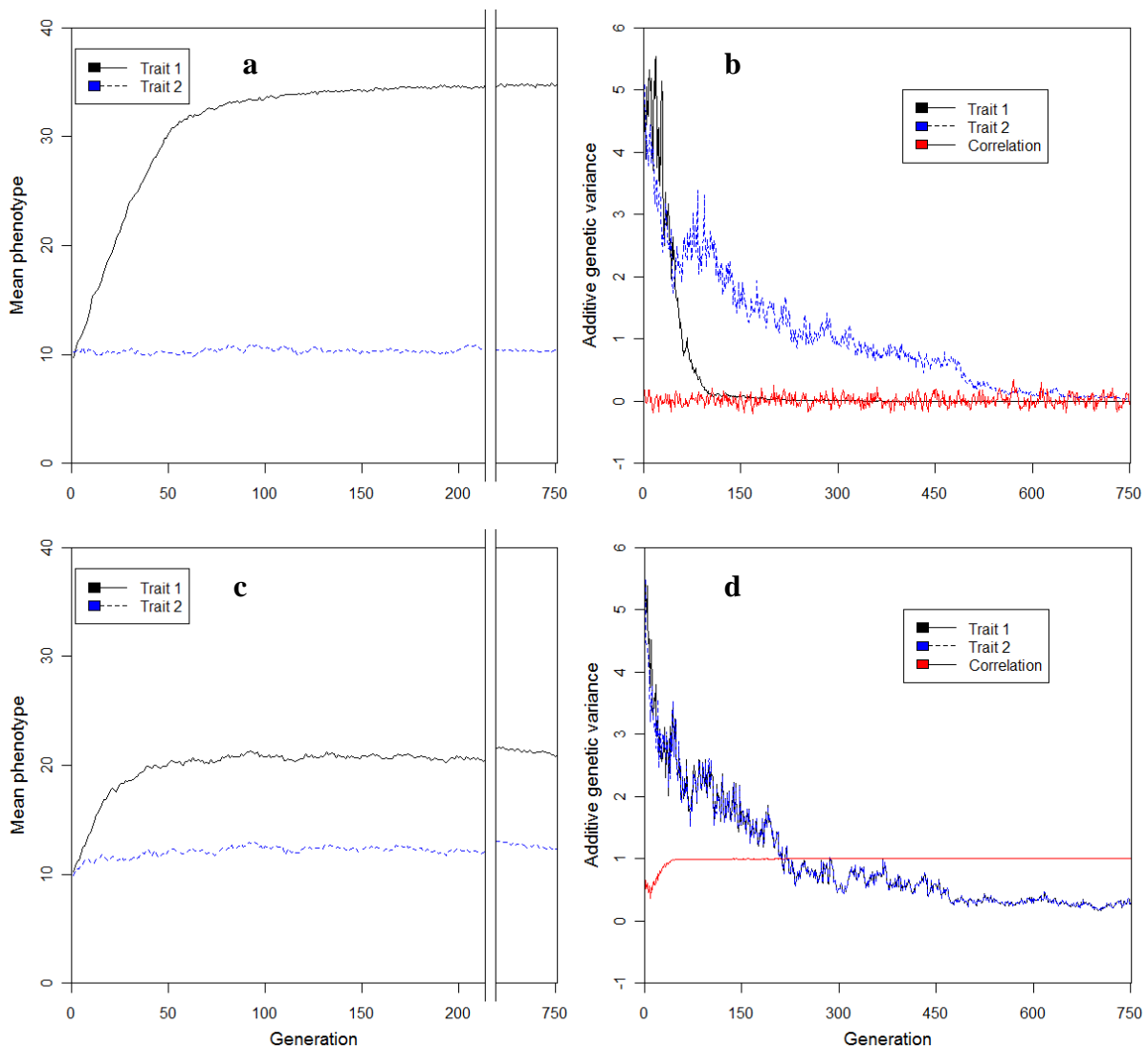

Figure 10 – The evolution of trait 1 and trait 2 with a modular (a, b) and a 40% hidden pleiotropic (c, d) GP map, example from one single run, mean phenotype (left) and additive genetic variance as well as genetic correlation (right).

In the case with the modular GP map the response in trait 1 lasts until the underlying additive genetic variance is approximately zero (fig. 11c and d), and trait 2 is maintained at the value 10 whereas its underlying additive genetic variance is reduced to almost zero, however,

slower than the additive genetic variance of trait 1. With the 100% hidden pleiotropic GP map, the responses are similar to the modular case (fig. 11e), however, with the additive genetic variance of trait 2 dropping equally fast as the additive genetic variance of trait 1 (fig. 11f). This is expected as these variances are underlain be the same loci. In both these cases the initial genetic correlation is approximately zero (fig. 11d and f). In the modular case it remains there throughout the time span, whereas in the hidden pleiotropic case the correlation increases, approximating unity after a few hundred generations. The hypothesis was that a correlation would build up and that this would have a constraining affect on the response. However, no constraining affect is observed (cf. fig. 11c and e). The correlation may result from only a very modest part of the underlying variance, as initially when most of the evolutionary change occurs and the level of additive genetic variance is high, the correlation is still low (11f). After most of the variance is depleted, a high genetic correlation does not matter. In addition, a certain displacement of trait 2 is possible without total cease of the response in trait 1 according to the fitness function (eq. 7). The magnitude of such a displacement is dependent on the strength of stabilizing and directional selection.

The responses in trait 1 in the scenarios of all the other GP maps in this experiment have values in-between those of the fully constrained and the modular map, the higher percentage of the pleiotropy being hidden, the higher the response (figs. 12 and 13a, and table 2). The differences in response between the first five B matrices are evident and about five times the environmental variance (V_E) in magnitude (range: 4.60 - 5.11). In comparison, the differences in response between the last three matrices (80% hidden, 100% hidden and modular) are very small. After 150 generations trait 1 has reached approximately its final value given all matrices (fig. 12). The level of additive genetic variance of trait 1 drops faster for the less constrained GP maps (fig. 13b).

The less pleiotropy is hidden the sooner trait 1 stops responding to selection, however, for the last three matrices (80% hidden, 100% hidden and modular) the responses behave very similarly (fig. 12). This means that with respect to selection limit a hidden pleiotropic GP map does not reduce evolvability compared to a modular GP map (table 2).
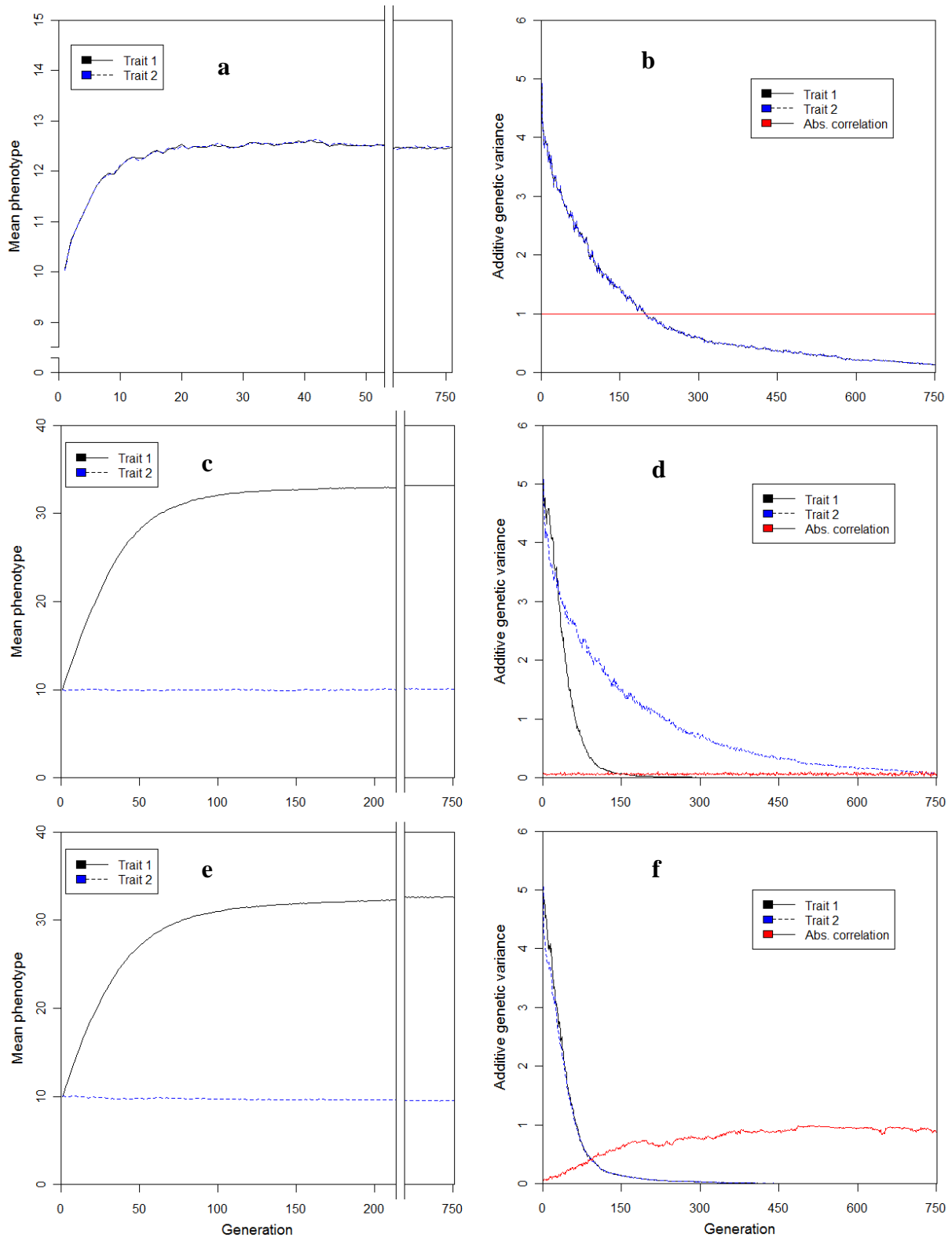
Figure 11 – The evolution of trait 1 and trait 2 with a fully constrained (a, b), a modular (c, d) and a 100% hidden pleiotropic (e, f) GP map (mean from 20 recursions), mean phenotype (left) and additive genetic variance as well as absolute values of the genetic correlation (right). (When the additive genetic variance of either trait approximates zero, the sample size for the calculation of the mean correlation decreases, because the correlation is not calculated in cases where the additive genetic variance of one of the traits equals zero.)
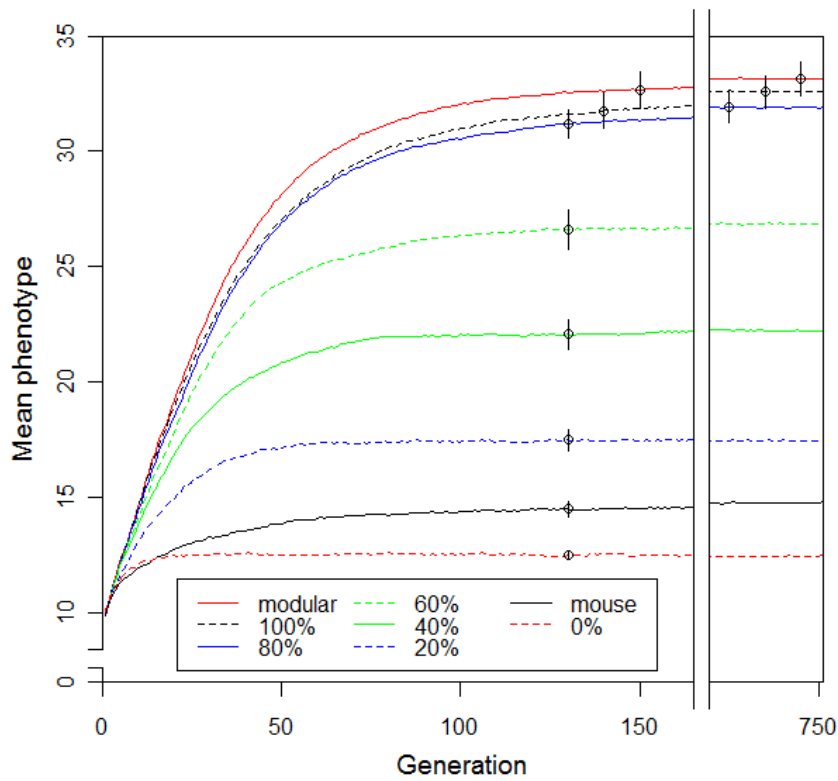
38

Figure 12 - The population mean of trait 1 is given as the mean value of 20 recursions. The results under the different GP maps are shown by the respective lines, the percentages indicating the degree of hidden pleiotropy. (Vertical bars are 95% confidence intervals.)
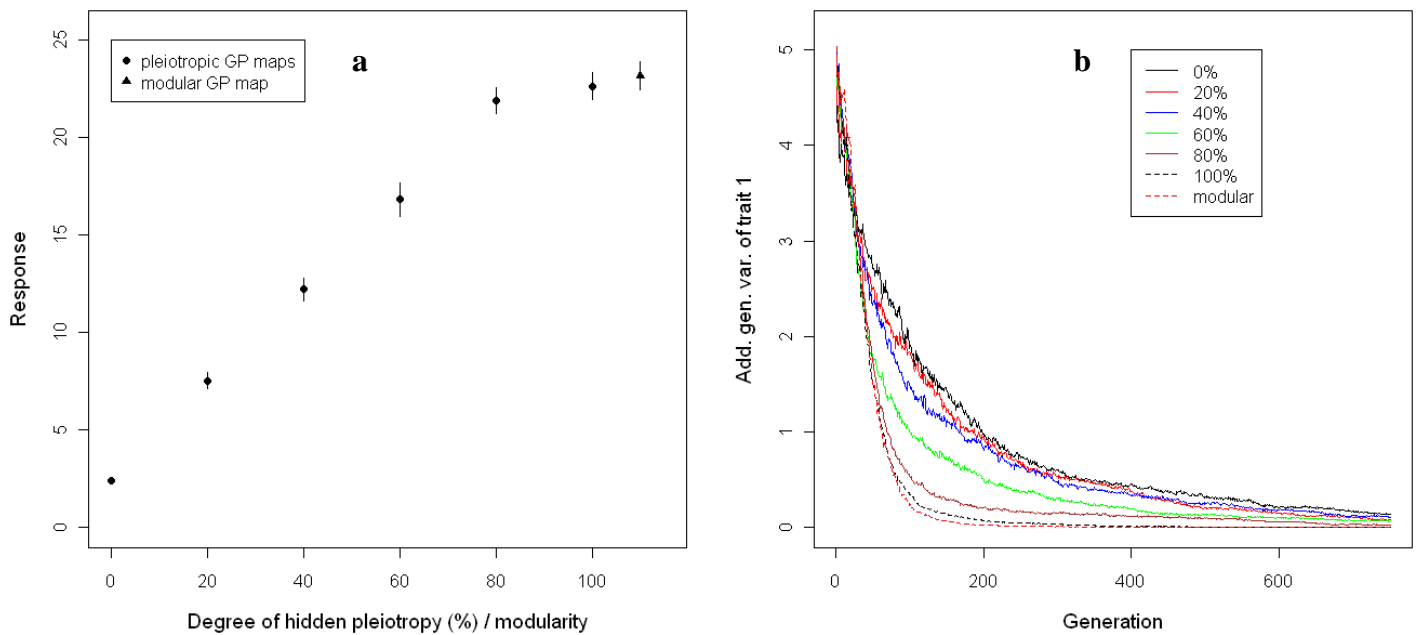

Figure 13 - a) The selection limit of trait 1 after 750 generations of selection (mean of 20 recursions) is given for GP maps of increasing degree of hidden pleiotropy, and compared to a modular map. (Vertical bars are 95% confidence intervals.) b) The additive genetic variance of trait 1 across generations for the different GP maps.

Table 2

GP map	Selection limit
0% hidden pleiotropy / fully constrained	2.42 (2.30, 2.54)
20% hidden pleiotropy	7.53 (7.12, 7.94)
40% hidden pleiotropy	12.21 (11.63, 12.79)
60% hidden pleiotropy	16.80 (15.95, 17.66)
80% hidden pleiotropy	21.89 (21.23, 22.54)
100% hidden pleiotropy	22.63 (21.92, 23.33)
(fully) modular	23.14 (22.41, 23.87)

The table shows the total response (mean and 95% CI) to directional selection in trait 1 when trait 2 is subject to stabilizing selection after 750 generations. The different rows represent the different genetic architectures.

4.1.2   Mouse B matrix

By comparison the estimated mouse femur-humerus B matrix resulted in a selection limit of 4.90 (with CI: (4.60, 5.19)), reached after about 200 generations, and almost reached after 100 generations (figs. 14a and 12). The absolute value of the correlation being close to one indicates high constraint for independent evolution in this GP map (fig. 14b).

As the distribution allelic effects with respect to direction in phenotype space (b_l) here were highly skewed (cf. section 3.2.2), this is probably not the most optimal B matrix based on the original in terms of evolvability, as according to the experiment on the number of loci (3.2.3), it is better with many small loci than a few large ones if the level of additive genetic variance is the same, cf. section 4.1.4. As initially $V_A = 2std^2 \sum_{j=1}^{L} b_{k,l}^2$ (eq. 9), the majority of the additive genetic variance is generated at the two first loci of the matrix, and as they are both quite synergistically pleiotropic this B matrix is apparently rather constrained. This means that the femur and humerus have limited possibilities for independent evolution, which is expected for homologous segments (Young et al. 2010).
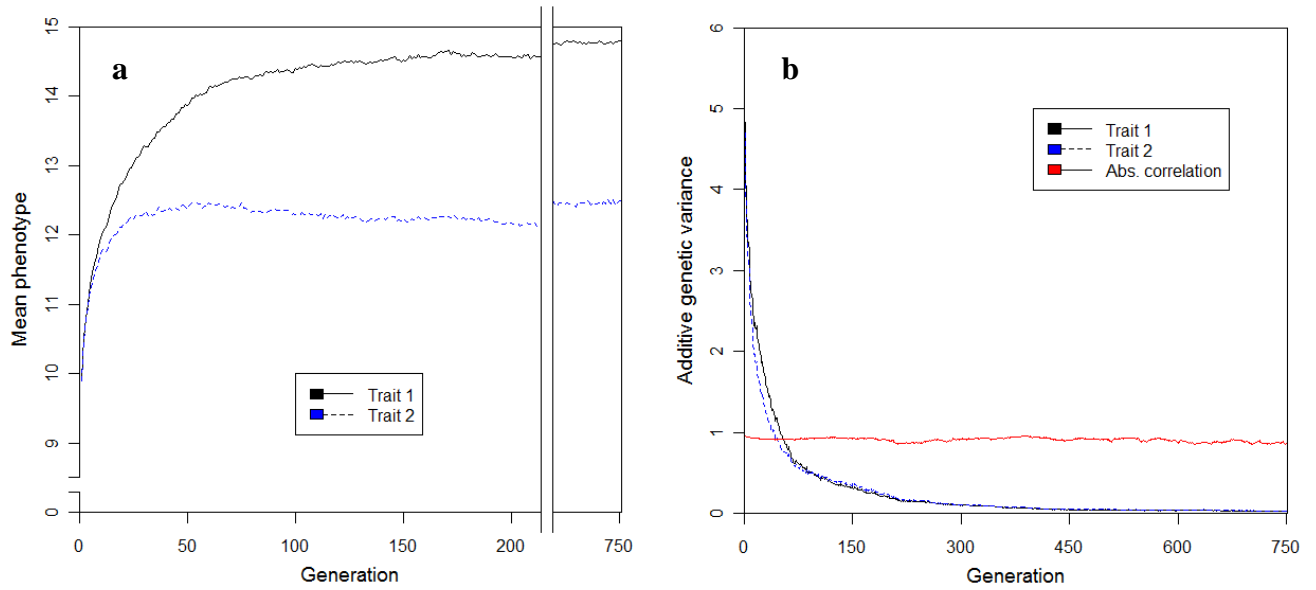
Figure 14 - The evolution of trait 1 and trait 2 with the mouse GP map (mean of 20 recursions), mean phenotype (a) and additive genetic variance (b) as well as absolute values of the correlation (b).

4.1.3 Short-term response

Here I compare the different GP maps with regard to the response in the first 20-100 generations, i.e. the short-term response. I found that the more of the pleiotropy is hidden the more generations it takes before trait 1 stops responding (fig. 15a). Initially, all GP maps give approximately equal response, the more constrained ones soon slowing down (fig. 15b). The first 20 generations of selection the evolution of trait 1 seems identical in the cases of the 100% hidden pleiotropic GP map and the modular GP map (fig. 15b). In these GP maps, the *a priori* prediction from the Lande equation for the first generation response is $\Delta \bar{z}_1 = G_{1,1} \beta_1 + G_{1,2} \beta_2 = 5 \cdot \frac{0.1}{1 - 0.02 \cdot 6} \approx 0.568182$, where $\beta_1 = \frac{s_1}{\bar{W}}$, $\beta_2 = \frac{-2 \cdot s_2(z_2 - {}^{opt}z_2)}{\bar{W}}$ and $\bar{W} = E[W] = 1 - s_2 \cdot (V[z_2] + (E[z_2] - {}^{opt}z_2)^2)$, as the initial expected G matrix is $G = \begin{bmatrix} 5 & 0 \\ 0 & 5 \end{bmatrix}$. The observed values are close to these (table 3).
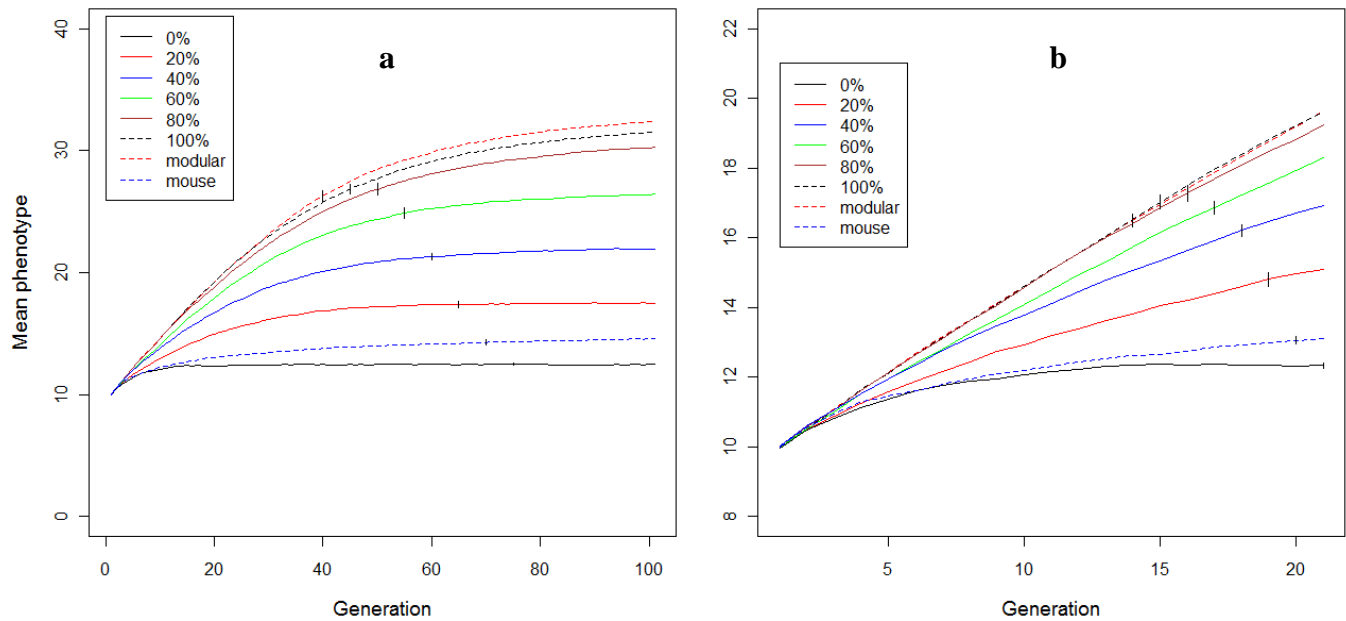
41

Figure 15 - Mean value of trait 1 for the different GP maps, across the first 100 (a) and 20 (b) generations. (Vertical bars are 95% CI.) Increased sample size ($c = 60$).

Table 3

GP map	Generation	Response
100% hidden pleiotropic		
	1	0.5246 (0.4765, 0.5728)
	5	0.5313 (0.5098, 0.5529)
	10	0.5124 (0.4964, 0.5284)
	15	0.5008 (0.4880, 0.5135)
	20	0.4813 (0.4693, 0.4932)
	40	0.4002 (0.3916, 0.4088)
	60	0.3207 (0.3133, 0.3281)
	80	0.2593 (0.2535, 0.2651)
	100	0.2151 (0.2106, 0.2197)
Modular		
	1	0.5388 (0.4856, 0.5921)
	5	0.5384 (0.5145, 0.5623)
	10	0.5112 (0.4966, 0.5259)
	15	0.4958 (0.4831, 0.5085)
	20	0.4826 (0.4702, 0.4951)
	40	0.4137 (0.4038, 0.4235)
	60	0.3334 (0.3266, 0.3401)
	80	0.2702 (0.2650, 0.2754)
	100	0.2235 (0.2192, 0.2278)

The table shows the cumulative response (mean and 95% CI) to directional selection in trait 1 divided by the number of generations elapsed, when trait 2 is subject to stabilizing selection. The different rows represent the different genetic architectures and after different numbers of generations.

42

With a smaller population size (N=50, c=40) the short-term evolvability of the modular and the 100% hidden pleiotropic GP map remained similar. After 5 generations the cumulative responses divided by the number of generations elapsed were 0.5112 (CI: (0.4715, 0.5508)) for the modular GP map and 0.4876 (CI: (0.4350, 0.5401) for the hidden pleiotropic map. After 100 generations the numbers were 0.1638 (CI: (0.1573, 0.1702)) and 0.1589 (CI: (0.1521, 0.1657)), respectively. The two GP maps also yielded similar results for the selection limit (after 750 generations, N=50, c=40) which were 16.78 (CI: (16.16, 17.39)) for the modular GP map and 16.35 (CI: (15.67, 17.03)) for the hidden pleiotropic map. Note however that except in the very short term (e.g. five generations) the response is significantly reduced in both GP maps for N=50 compared to N=200 (cf. table 2 and table 3). For the cumulative evolvability this is true already after 20 generations of selection. When the number of loci is reduced to 4 (L=4, N=200, c=40) such that expected initial $G = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$ for both the modular and the hidden pleiotropic GP map, the selection limit (after 350 generations) and the short term cumulative response are still approximately equal between these two GP maps, the modular having slightly larger estimates, as in the other cases.

4.1.4   The effect of number of loci

Comparing the response to selection in GP maps comprising 2 and 50 loci with the same initial G matrix, I found that the total response after 750 generations of selection (fig. 16a) was approximately 3.6 times higher in the case of 50 loci (Selection limit = 44.47, CI: (43.53, 45.41)) than in the case of two loci (Selection limit = 12.32, CI: (11.23, 13.40)). In both cases the GP map was fully modular and the initial G matrix was equal, meaning that also the amount of genetic variance for trait 1 was equivalent (fig. 16b). The following mean G matrices resulted:

2 loci:

Initially: $G = \begin{bmatrix} 4.971 & 0 \\ 0 & 0 \end{bmatrix}$. After 750 generations: $G = \begin{bmatrix} 1.626e-05 & 0 \\ 0 & 0 \end{bmatrix}$.

50 loci:

Initially: $G = \begin{bmatrix} 4.837 & 0 \\ 0 & 0 \end{bmatrix}$. After 750 generations: $G = \begin{bmatrix} 0.003371 & 0 \\ 0 & 0 \end{bmatrix}$.
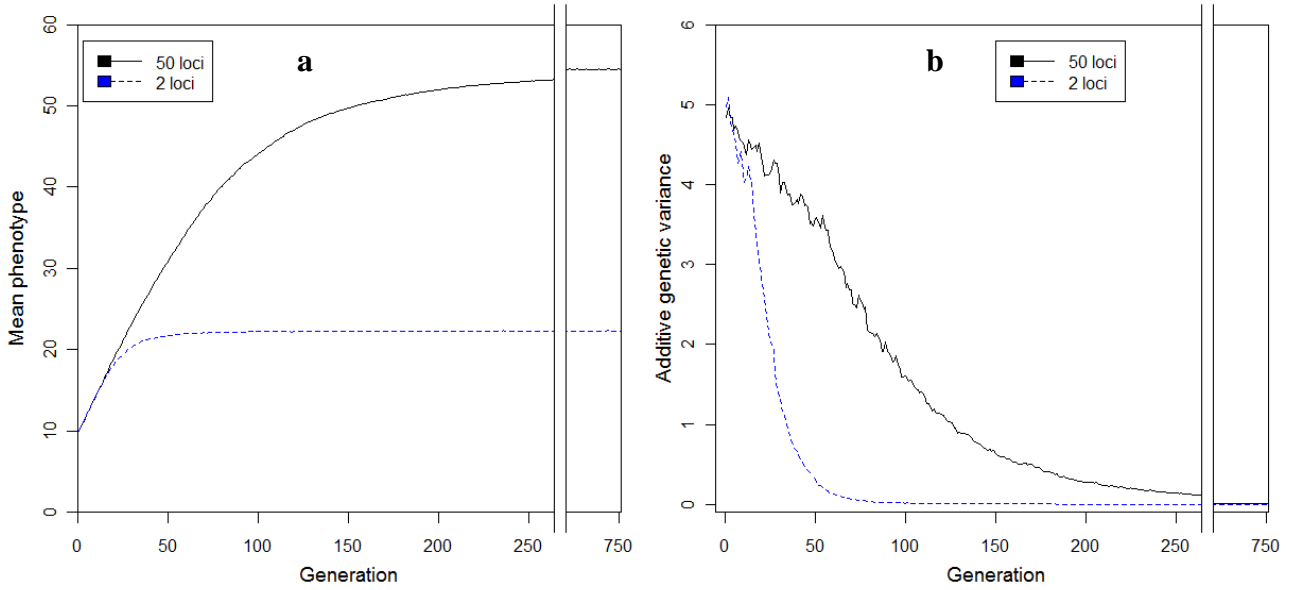
Figure 16 - Evolution of trait 1 (a) with a GP map of two loci with all $b_{1,l}$ equal to 2.236068 *vs.* one with 50 loci with all $b_{1,l}$ equal to 0.4472136, and the amount of additive genetic variance of trait 1 (b).

The reason why the selection limit of the 50 loci GP map (b) was much higher it was for the 2 loci GP map (a) is apparently because as $z_1 = 10 + B_1 \cdot y + e_1 = \sum_{j=1}^{L} b_{1,l} \cdot y_l + e_1$, then for some maximal value d of all y_l (when alleles have gone to fixation), and all $b_{1,l}$ being equal $E[z_1] = 10 + L \cdot b_{1,l} \cdot d$, making $E[z_1]$ in (a) become $10 + 2 \cdot 2.236068 \cdot d = 10 + 4.472136d$, and in (b) $E[z_1] = 10 + 50 \cdot 0.4472136 \cdot d = 10 + 22.36068d$. In this case if $E[z_1]$ in (a) is e.g. 20 then $E[z_1]$ in (b) would be 60, resulting in the expected response $= E[z_1] - 10$ five times larger in (b) than in (a). This also follows from the fact that $b_{1,l}$ in (a) is only five times larger than in (b), whereas in (b) L is 25 times larger than in (a). This means that if the current experiment results in equal mean allelic values in both (a) and (b) when all genetic variance is depleted, then the total response in (b) will be five times larger than in (a).

Would one rather have varied the std value than the values of the $b_{1,j}$'s to yield equal initial G matrix, $G = \begin{bmatrix} 5 & 0 \\ 0 & 0 \end{bmatrix}$ in (a) and (b), then if we let all $b_{1,j}$ equal a number b, $5 = 2std^2 \cdot L \cdot b^2$. For simplicity we let $b = 1$ and get $5 = 2std^2 \cdot L$. Then in (a) $std = \frac{\sqrt{5}}{2}$ and in (b) $std = \frac{\sqrt{5}}{10}$. As the standard deviation of the allelic values (std) in (a) is five times larger than in (b), and L is 25 times larger in (b) than in (a), I would expect this scenario to yield similar results.

44

This implies that even if the initial G matrix is the same, the total response in the long term is larger when the underlying GP map comprises many loci with small effects than if it consists of a few loci with large effects. This is important to have in mind when constructing different B matrices, and when comparing the response to selection of different GP maps.

4.1.5   Alternative allelic distributions

In the biallelic setting the modular GP map yielded an almost immediate fast response (fig. 17a), whereas the response given the pleiotropic GP map was delayed for some tens of generations (the delay was highly variable). In the setting with alleles drawn from a normal distribution the two GP maps yielded very similar responses (fig. 17b).
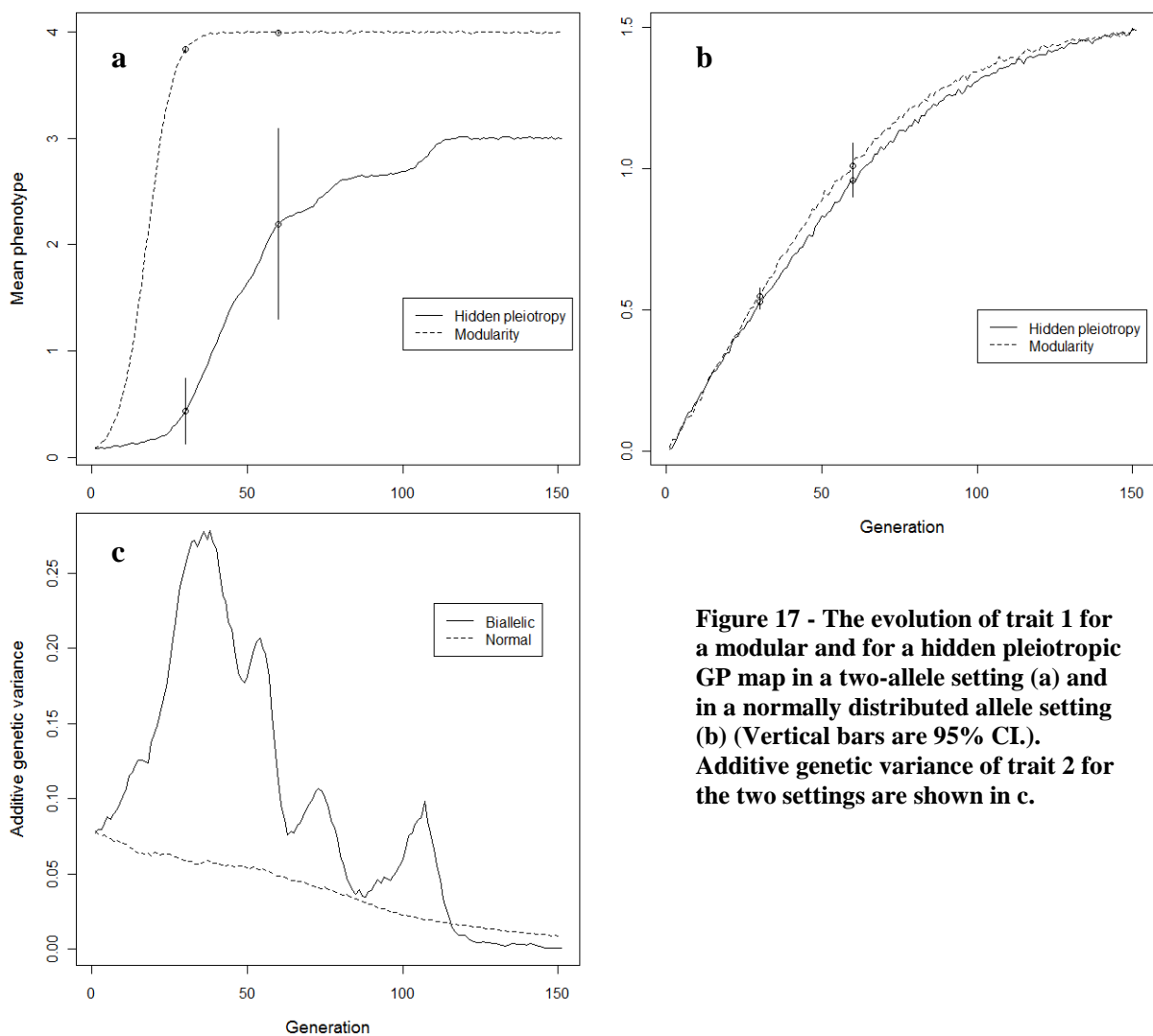

Figure 17 - The evolution of trait 1 for a modular and for a hidden pleiotropic GP map in a two-allele setting (a) and in a normally distributed allele setting (b) (Vertical bars are 95% CI.). Additive genetic variance of trait 2 for the two settings are shown in c.

Note that the adaptive dynamics of trait 1 in the two settings are quite different. As explained by Baatz and Wagner (1997), the reduction in the evolutionary response of trait 1 is linked to the increase in the variance of trait 2. As can be seen in figure 17c, an increase in the additive genetic variance of trait 2 takes place in the biallelic setting, but not in the normal distribution setting. It seems that the hidden pleiotropic GP map does not suffer from the "adaptive inertia" found in Baatz and Wagner (1997) when a continuum of alleles with different effect sizes are present, suggesting that adaptive inertia caused by hidden pleiotropy is not a common phenomenon of phenotypic evolution.

4.1.6   Mutation-based simulations

After 1000 generations of mutation and selection the modular GP map gave a slightly higher total response than the pleiotropic one, $106.36V_E$ (CI: (100.86, 111.85)) and $98.71V_E$ (CI: (94.68, 102.75) respectively. Baatz and Wagner (1997) showed that the degree of constraint from hidden pleiotropy was dependent on the relationship between the strength of directional and stabilizing selection, weak directional and strong stabilizing selection being highly constraining. As I have used a fitness function with linear directional selection and quadratic stabilizing selection, for a single mutation to be beneficial it must be under a certain value and that value depends on the strength of directional and stabilizing selection, as explained above. According to the fitness function (eq. 7), $W = 1 + s_1(z_1 - \bar{z}_1) - s_2(z_2 - {}^{opt}z_2)^2$, assuming the population mean is at the optimum trait 2 value, a mutation effect of size δ is beneficial (with the hidden pleiotropic GP map) if $s_1 \cdot \delta - s_2 \cdot \delta^2 > 0$. The standard deviation of mutational effect size in this case was $mstd = 0.5$, making $s_1 \cdot \delta - s_2 \cdot \delta^2 = 0.045 > 0$. Most mutations would thus be expected to be immediately beneficial, given the assumptions made. If, however, $mstd$ is increased to e.g. 6, then $s_1 \cdot \delta - s_2 \cdot \delta^2 = -0.12 < 0$. In that case most mutations are expected to be initially detrimental. Indeed, when I repeated the experiment with $mstd = 6$, I found a much larger relative difference in evolutionary response between the modular and the pleiotropic GP maps, 5915.31 (CI: (5826.57, 6004.05)) and 2094.21 (CI: (2014.31, 2174.11), respectively. In the case with $mstd = 0.5$, the modular GP map yielded approximately only an 8% higher response than the hidden pleiotropic GP map, whereas when $mstd = 6$ the difference was approximately 180%.

If the criterion $s_1 \cdot \delta - s_2 \cdot \delta^2 = -0.12$ was to be achieved by changing the strength of stabilizing selection (s_2) rather than the mutational standard deviation ($mstd$), the

corresponding value when $mstd = 0.5$ would be $s_2 = 0.68$. This is much higher than the value I have been using in most of the experiments (0.02). For the modular GP map this gives a total response after 1000 generations of 111.98 (CI: (107.73, 116.24)), which is approximately 810% of the response with the hidden pleiotropic GP map, which was 13.84 (CI: (12.07, 15.62)). In this case hidden pleiotropy is highly constraining.

4.1.7   Even B matrix

The question here was whether it matters what the specific directions of the hidden pleiotropic effects are for the evolvability. The selection limit of the even B matrix was slightly (about 3%) higher than the one of the reversed even B matrix. These B matrices yielded somewhat lower selection limits than the modular and 100% hidden pleiotropic ones (about 6-12% lower after 750 generations of selection, see tables 2 and 4).

For the short-term response, initially no significant difference is observed neither between the two even matrices nor between these and the modular and hidden pleiotropic one. However, after about 5 to 15 generations a small difference is seen in the favor of the modular and hidden pleiotropic GP maps (see tables 3 and 5), and from about that point on there is a tendency for the even B matrix to yield a slightly higher response than the reversed even B matrix.

Table 4

GP map	Selection limit
Even B	21.39 (20.61, 22.17)
Reversed even B	20.71 (19.93, 21.50)

The table shows the response (mean and 95% CI, $c = 20$) to directional selection in trait 1 when trait 2 is subject to stabilizing selection. The different rows represent the different genetic architectures.

Table 5

GP map	Generation	Response
Even B		
	1	0.5387 (0.4887, 0.5888)
	5	0.5095 (0.4868, 0.5322)
	10	0.4942 (0.4794, 0.5090)
	15	0.4819 (0.4676, 0.4962)
	20	0.4659 (0.4527, 0.4791)
	40	0.3793 (0.3701, 0.3886)
	60	0.2938 (0.2874, 0.3002)
	80	0.2341 (0.2293, 0.2389)
	100	0.1931 (0.1893, 0.1969)
Reversed even B		
	1	0.5262 (0.4816, 0.5707)
	5	0.5176 (0.4986, 0.5367)
	10	0.4911 (0.4798, 0.5023)
	15	0.4803 (0.4699, 0.4907)
	20	0.4599 (0.4496, 0.4702)
	40	0.3695 (0.3595, 0.3794)
	60	0.2885 (0.2807, 0.2963)
	80	0.2313 (0.2253, 0.2373)
	100	0.1907 (0.1860, 0.1955)

The table shows the cumulative response (mean and 95% CI, $c = 60$) to directional selection in trait 1 divided by the number of generations elapsed, when trait 2 is subject to stabilizing selection. The different rows represent the different genetic architectures and after different numbers of generations.

As the even B matrices are also 100% hidden pleiotropic (with some modular loci) because they initially yield no correlations, it is natural to compare them with the 100% hidden pleiotropic B matrix (B6) of the experiment in section 3.2.1. The latter had a 5.79% higher selection limit than the even B matrix and a 9.24% higher selection limit than the reverse even B. The even B gave a selection limit that was 3.26% higher than the selection limit of the reversed even B matrix. The corresponding relationships between the sums of the absolute values of the entries of B that map to trait 1 were respectively 9.25%, 14.88% and 5.15%.

Even if these latter ratios are not exactly equal to those of the selection limits, it is natural to ask whether the selection limit can be predicted by the sum of the absolute values of the entries in the B matrix mapping to trait 1. It has certainly been shown that the G matrix is not sufficient in these cases. To test this hypothesis I have created two new B matrices yielding equal expected initial G matrix but where one of them has entries of the B matrix mapping to trait 1 that sum up to a 25% higher value then those of the other. They are both 100% hidden

pleiotropic. I compared their evolutionary responses using the same parameter values as for the other experiments on hidden pleiotropy (section 3.2.1). Note that the expected initial heritability (h^2) here is 99%. B matrices:

Regular B matrix $= \begin{bmatrix} 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 \\ 5 & 5 & 5 & 5 & -5 & -5 & -5 & -5 \end{bmatrix}$ $where \ \sum_{l=1}^{L} b_{1,l} = 40, \sum_{l=1}^{L} b_{1,l}^2 = 200$

Irregular B matrix $= \begin{bmatrix} 1 & 1 & 1 & 1 & 7 & 7 & 7 & 7 \\ 1 & 1 & -1 & -1 & 7 & 7 & -7 & -7 \end{bmatrix}$ $where \ \sum_{l=1}^{L} b_{1,l} = 32, \sum_{l=1}^{L} b_{1,l}^2 = 200 \ .$

The regular B matrix yielded a selection limit of 92.11 with CI: (89.17, 95.05), whereas the irregular one yielded 72.86 with CI: (70.51, 75.21), the selection limit of the regular one thus being 26.42% higher that of the irregular B matrix. This is close to the predicted 25%. It thus seems likely that the sum of the absolute values of the B matrix entries mapping to the focal trait gives a good prediction of the selection response. In geometrical terms these values are the lengths of the projections of the vectors $\boldsymbol{b_l}$ (fig. 8) onto the trait 1 axis. This could be generalized to the hypothesis that it is the sum of these projections onto the axis of any direction of selection ($\boldsymbol{\beta}$) that predicts the selection limit. This implies that when this sum is unequal among different GP maps, the G matrix alone does not give a good measure of evolvability.

4.1.8   Partial hidden pleiotropy *vs*. partial modularity

Here I investigated whether a partially modular GP map enables a higher response than a partially hidden pleiotropic GP map with the same initial G matrix. The partially modular GP map had a higher total response after 350 generations of selection (at which point the remaining additive genetic variance of trait 1 was less then 10% of the initial value, however, in the modular case less remained than in the pleiotropic case, 0.02 and 0.34 respectively) (table 6, fig 18). For the short-term evolvability the difference between the two architectures is less evident (table 7), the modular GP map evolving slightly faster than the pleiotropic one after some generations.

Table 6

GP map	Selection limit
Partially hidden pleiotropic	12.81 (12.17, 13.45)
Partially modular	17.57 (16.71, 18.42)

The table shows the response (mean and 95% CI) to directional selection in trait 1 when trait 2 is subject to stabilizing selection. The different rows represent the different genetic architectures.

Table 7

GP map	Generation	Response
Partially hidden pleiotropic		
	1	0.4322 (0.3634, 0.5011)
	5	0.3761 (0.3507, 0.4014)
	10	0.3531 (0.3309, 0.3754)
	15	0.3434 (0.3235, 0.3633)
	20	0.3328 (0.3133, 0.3523)
	40	0.2610 (0.2460, 0.2760)
	60	0.1975 (0.1857, 0.2092)
	80	0.1546 (0.1461, 0.1631)
	100	0.1260 (0.1188, 0.1331)
Partially modular		
	1	0.4151 (0.3283, 0.5018)
	5	0.3638 (0.3349, 0.3926)
	10	0.3547 (0.3339, 0.3755)
	15	0.3332 (0.3135, 0.3529)
	20	0.3178 (0.2999, 0.3358)
	40	0.2674 (0.2526, 0.2823)
	60	0.2221 (0.2104, 0.2339)
	80	0.1859 (0.1770, 0.1948)
	100	0.1572 (0.1496, 0.1648)

The table shows the cumulative response (mean and 95% CI) to directional selection in trait 1 divided by the number of generations elapsed, when trait 2 is subject to stabilizing selection. The different rows represent the different genetic architectures and after different numbers of generations.
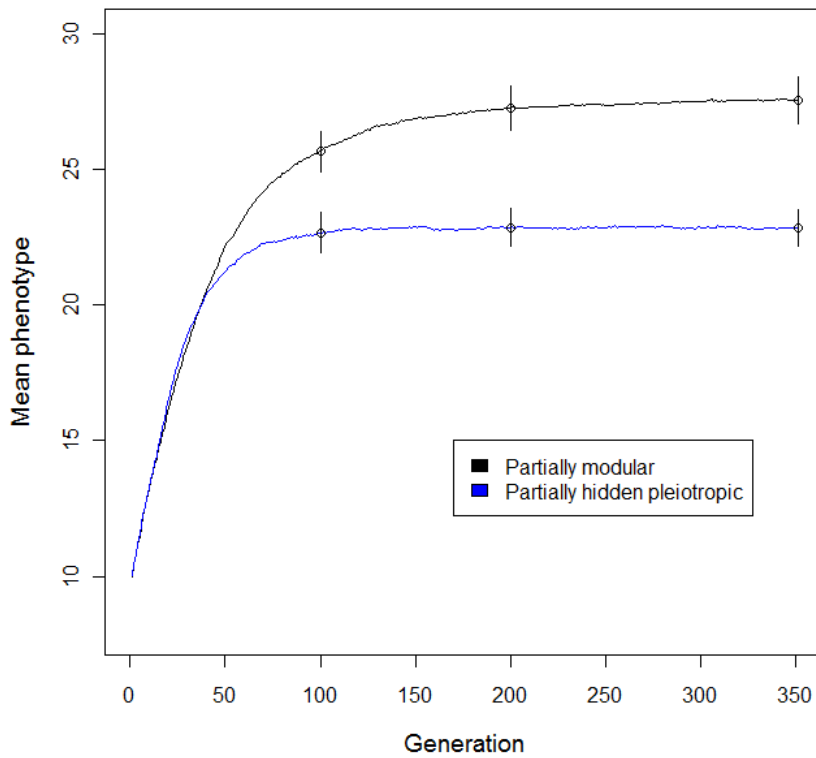
Figure 18 – The evolution of trait 1 with a partially modular and a partially hidden pleiotropic GP map. (Vertical bars are 95% CI.)

4.1.9   Linkage disequilibrium

When I tested for effects of linkage disequilibrium on the response in trait1, I found that the response when the GP map had no effects on trait 2 was only slightly larger than it was when the GP map was modular (table 8, fig. 19).

Table 8

GP map	Selection limit
No effects on trait 2	24.21 (23.26, 25.17)
Modular	23.14 (22.41, 23.87)

The table shows the total response (mean and 95% CI) to directional selection in trait 1 when trait 2 is subject to stabilizing selection after 750 generations. The different rows represent the different genetic architectures.
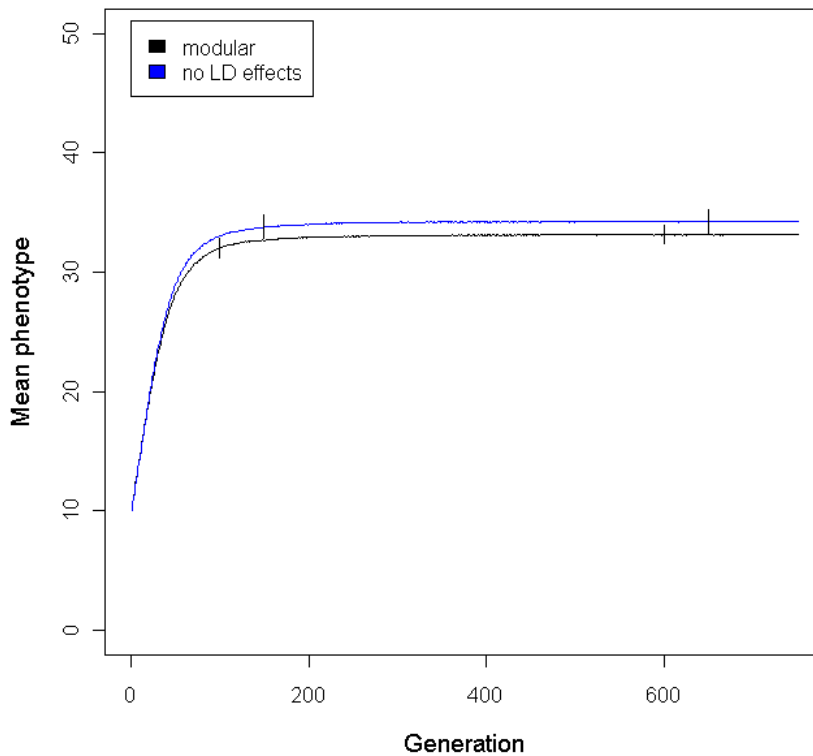
Figure 19 – The evolution of trait 1 in the case of a modular GP map and in the case of a GP map with no effects on trait 2 (mean from 20 recursions). (Vertical bars are 95% confidence intervals.)

This difference should be due to linkage disequilibrium. LD effects are thus not important.

4.1.10   Effects of the strength of stabilizing selection

When the stabilizing selection becomes relatively strong, $s_2 = 0.08$ (log(s_2) = -2.526), there is a small difference in selection limit of trait 1 between the modular GP map (23.75V_E) and the 100% hidden pleiotropic GP map (21.06V_E). In comparison, at this level of stabilizing selection the response with a fully constrained GP map is 0.59V_E. In the case of 100% hidden pleiotropy the response seems to be an approximately linear function of log(s_2) (fig. 20). In the case of weaker stabilizing selection ($s_2 = 0.04$, $s_2 = 0.02$, $s_2 = 0.01$ or log(s_2) = -3.219, log(s_2) = -3.912, log(s_2) = -4.605) the difference between the response in trait 1 of the modular map and the 100% hidden pleiotropy map was very small (fig. 20, table 9).

For the fully constrained GP map the evolutionary short-term response decreased approximately linearly with increasing strength of stabilizing selection, as was the case with

the selection limit. However, for both the modular and the 100% hidden pleiotropic GP map
this relationship was quite different. After 20 generations of selection the mean rate of
evolution increases somewhat with the strength of stabilizing selection for these GP maps
(table 10). The values for the modular map tend to be slightly higher than those of the
pleiotropic map. However, there is no significant difference between the modular and the
hidden pleiotropic GP map at any level of stabilizing selection.

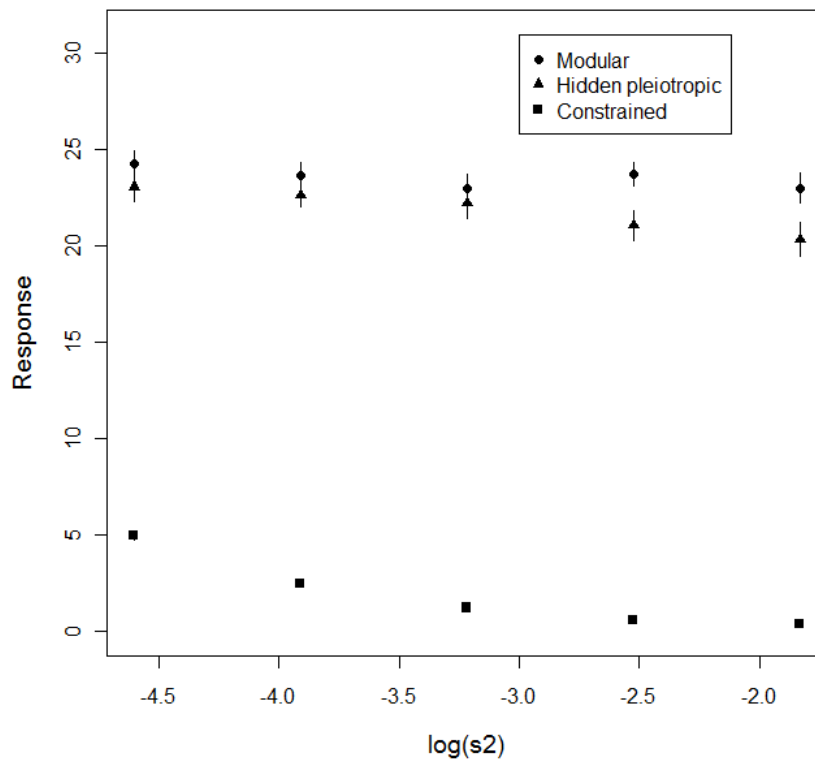**Figure 20 – The y-axis gives the selection limit of trait 1 after 750 generations at different levels of
stabilizing selection on trait 2 and under different GP maps. The x-axis contains the natural logarithm of
the selection coefficient of trait 2 (s_2), cf. the fitness function (eq. 7). The response is in units of
environmental variance (V_E). (Vertical bars are 95% confidence intervals.)**

Table 9

GP map	s_2	Selection limit
100% hidden pleiotropic		
	0.01	23.07 (22.32, 23.81)
	0.02	22.63 (22.06, 23.19)
	0.04	22.22 (21.42, 23.01)
	0.08	21.06 (20.30, 21.82)
	0.16	20.35 (19.47, 21.23)
Modular		
	0.01	24.31 (23.64, 24.98)
	0.02	23.64 (22.95, 24.33)
	0.04	22.97 (22.22, 23.72)
	0.08	23.75 (23.13, 24.36)
	0.16	23.00 (22.23, 23.78)
Fully constrained		
	0.01	4.96 (4.78, 5.14)
	0.02	2.47 (2.36, 2.59)
	0.04	1.23 (1.13, 1.33)
	0.08	0.59 (0.51, 0.67)
	0.16	0.38 (0.31, 0.45)

The table shows the total response (mean and 95% CI) to directional selection in trait 1 when trait 2 is subject to stabilizing selection after 750 generations. The different rows represent the different genetic architectures and levels of stabilizing selection (s_2).

Table 10

GP map	s_2	Response
100% hidden pleiotropic		
	0.01	0.4715 (0.4532, 0.4898)
	0.02	0.4632 (0.4424, 0.4841)
	0.04	0.4712 (0.4440, 0.4983)
	0.08	0.4995 (0.4716, 0.5274)
	0.16	0.5213 (0.4919, 0.5507)
Modular		
	0.01	0.4909 (0.4725, 0.5092)
	0.02	0.4990 (0.4684, 0.5295)
	0.04	0.5035 (0.4826, 0.5243)
	0.08	0.5622 (0.5369, 0.5875)
	0.16	0.5746 (0.5424, 0.6067)
Fully constrained		
	0.01	0.2200 (0.2117, 0.2284)
	0.02	0.1205 (0.1128, 0.1282)
	0.04	0.0617 (0.0573, 0.0661)
	0.08	0.0298 (0.0255, 0.0340)
	0.16	0.0179 (0.0140, 0.0219)

The table shows the cumulative response (mean and 95% CI) to directional selection in trait 1 for the first 20 generations of selection divided by 20. The different rows represent the different genetic architectures and levels of stabilizing selection (s_2).

4.1.11 Pleiotropy as a source of variation

The question here was whether an intermediate level of pleiotropy can be beneficial even though it generates correlations, when it contributes to the additive genetic variance of the focal trait. When the level of pleiotropy reaches about 40% the selection limit drops in a linear fashion (fig. 21a). However, it is maximized at an intermediate level of pleiotropy, that is, when the percentage of pleiotropic loci is in the area 24 − 36 %. For short-term initial evolutionary rate, trait 2 had stopped responding at least after 10 generations of selection. This means equilibrium between the opposing selective forces had been reached, and I compared the mean rate of evolution from generation 11 to 21. The same trend as for the selection limit is present here as well, but it is not as clear (fig. 21b). The single highest estimated value was for B matrix 5, corresponding to 16% pleiotropy, as predicted by Hansen (2003). However, all GP maps in the range 8-40% pleiotropy had similar responses and cannot be clearly distinguished based on this experiment.
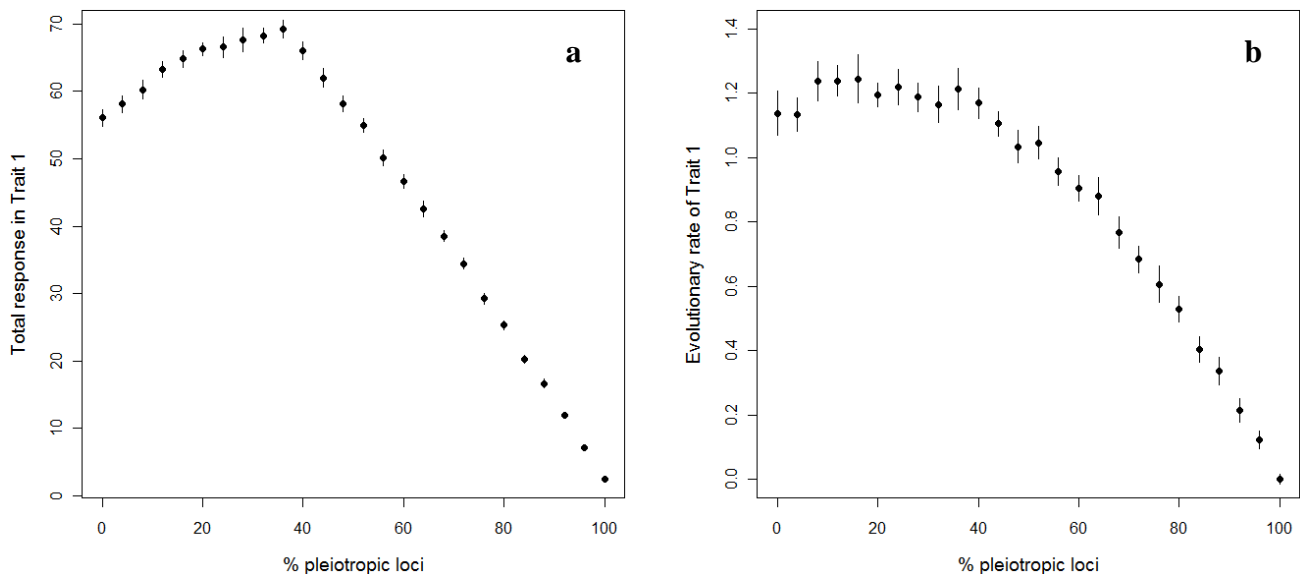

Figure 21 - The selection limit of trait 1 after 350 generations (a) and mean per generation response of 10 consecutive generations after 10 generations of selection (b) (mean of 20 recursions) are given for each GP map. (Vertical bars are 95% confidence intervals.)

4.1.12   Predictive value of the conditional evolvability

Her I tested the predictive value of the Hansen (2003) *conditional evolvability* where $\Delta \bar{z}_1 = G_{Z_1|Z_2} \cdot \beta_1 = \left(G_{1,1} - \frac{G_{1,2}^2}{G_{2,2}} \right) \cdot \frac{s_1}{\bar{w}}$. As the selective equilibrium was reached after the first few generations (<20) (fig. 22), I analyzed predictions about the response in trait 1 starting after 20 generations of selection. Both the Lande equation, which included the indirect selection term (the bivariate Lande, with estimated z_2 term), and the conditional evolvability gave good predictions of the evolution of trait 1, when stabilizing selection on trait 2 was present. The GP map then included 36% synergistic pleiotropy, and the rest of the loci were modular (fig. 23). The univariate Lande equation (disregarding the effects of indirect selection) gave highly inaccurate predictions. When a GP map with 68% pleiotropy was used, the bivariate Lande equation estimator gave slightly worse predictions whereas the conditional evolvability remained highly accurate. The unconditional additive genetic variance (evolvability) in the case with 68% pleiotropy started out and remained higher than it did in the case with 36% pleiotropy. For the conditional variance this relationship was reversed. For both GP maps the unconditional and the conditional variance were reduced in a similar manner across evolutionary time. The unconditional variance was always higher than the conditional variance (fig. 23). The GP maps with only antagonistic pleiotropy yielded similar results.
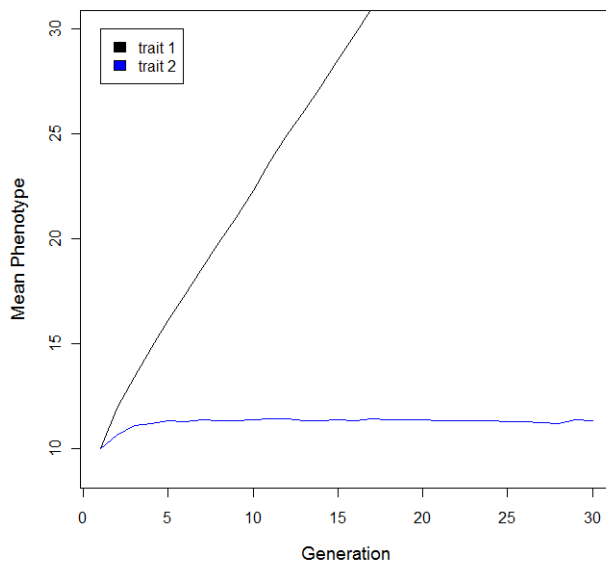

Figure 22 – The evolution of trait 1 and trait 2 with a GP map of 36% of the loci being synergistically pleiotropic. Trait 2 has reached its total displacement from optimum after about five generations, so the selective equilibrium is obtained.
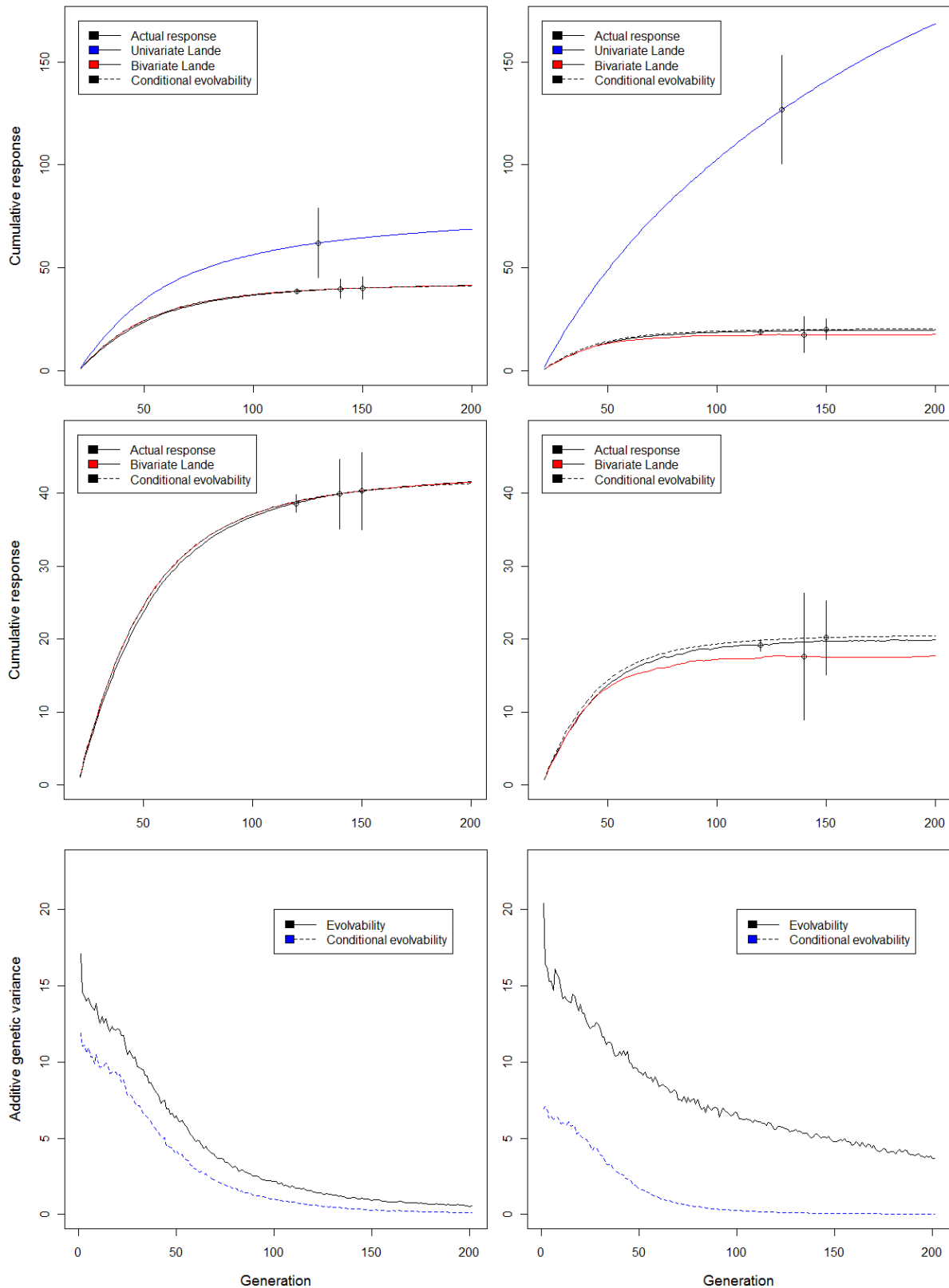
Figure 23 - The cumulative response (mean of 20 recursions) in trait 1 and the mean of its respective predicted values from different estimators with a GP map of 36% pleiotropy (left) and a GP map of 68% pleiotropy (right) with only synergistic effects (95% CI for the actual response and 95% PI (prediction intervals) for the estimates are shown as vertical bars.). Unconditional and conditional evolvabilities of the respective GP maps are shown in the bottom row (These include the first 20 generations, and thus the phase before equilibrium is reached.).

5   Discussion

According to the Lande equation, the per generation change in population means of multiple quantitative traits ($\Delta \bar{z}$) equals the product of the additive genetic variance-covariance matrix ($\boldsymbol{G}$) and the selection gradient ($\boldsymbol{\beta}$). The selection gradient is a linear representation of selection in terms of change in fitness per change in phenotype, and the G matrix expresses how much heritable variation there is in each trait (z_k), and how much of this variation is independent of the other traits. As this representation separates selection from variation, the ability to respond to selection is reflected in the G matrix. Whether the G matrix is a sufficiently good measure of evolvability is however not trivial (Houle 1991; Gromko 1995; Baatz and Wagner 1997; Pavlicev and Hansen 2011). The Lande equation predicts evolutionary change from one generation to the next, but how the G matrix changes over time, and thus how the response changes, depends on the way in which the genetic variation is expressed as phenotypic variation. In other words, evolvability depends on the genotype-phenotype map (Wagner and Altenberg 1996). A crucial aspect of the GP map that can affect evolvability is its pleiotropic structure, as pleiotropy is the major source of genetic correlations and has thus the potential to impede independent evolution of traits. If we assume the pleiotropy at each locus is a stable property of the GP map, the genetic component ($\boldsymbol{x}$) of the phenotype can be modeled as the product of a matrix ($\boldsymbol{B}$), describing the GP map, and a vector ($\boldsymbol{y}$) of allelic values (Wagner 1989). It has been criticized that such linear representations of the GP map are not realistic and cannot account for all types of evolutionary change (Polly 2008). However, they can be good approximations, and are useful for testing hypotheses of how different factors affect evolutionary dynamics (Hansen 2008). I have used the model of Wagner (1989) in simulation experiments to explore a variety of different GP maps in order to learn about the effects of the underlying pleiotropic structure on the evolvability of quantitative traits. A scenario was created where a population of individuals with two traits experienced conflicting selection pressure, as one trait was under directional selection and the other was under stabilizing selection. To find out whether the underlying pleiotropic structure matters if the G matrix is given, I have compared GP maps that yield the same expected (average) initial G matrix. The main result is that $\boldsymbol{G}$ is in general a good indicator of evolutionary potential and constraint (Roff 2007), and pleiotropy that does not contribute to the genetic covariance has only subtle effects on evolvability. An important finding is that both the Lande equation and the

conditional evolvability are good predictors of evolutionary response across multiple generations, despite extensive pleiotropy in the underlying GP map. According to Roff (2007), quantitative genetic theory does not predict well when selection and genetic correlation are of opposite signs. Here, however, the contrary was found. The conditional evolvability proved even more precise than the Lande equation (with estimated β_2), and it has the great advantage of not demanding information about the strength of stabilizing selection. This result, as well as other findings of the study, shows that the strength of stabilizing selection is not determining the evolutionary outcome either. It was primarily the selection limit (total evolutionary change achievable) that was affected by the underlying GP map. Here the partially modular structure proved advantageous when genetic correlations were present, and the number of loci as well as the absolute effect sizes in the GP map were highly determinative despite a constant initial G matrix.

The main result from the experiments on hidden pleiotropy was that the response to directional selection is not significantly impaired by stabilizing selection on another trait when these two traits are only connected by hidden pleiotropy, given only standing genetic variance and initially normally distributed allelic effects. This holds for both short- and long-term evolution (selection limit), for many as well as very few loci, and for different population sizes. The result is, however, conditional on the stabilizing selection not being too strong compared to directional selection. My hypothesis was that changes in allele frequencies would generate correlations strong enough to constitute a constraint. This hypothesis was rejected here. However, as other studies have shown (Slatkin and Frank 1990; Baatz and Wagner 1997; Griswold 2006), this is not valid for all distributions of allelic effects. In non-normal distributions the underlying pleiotropic structure of the GP map can matter. Gromko (1995) showed that different pleiotropic structures of the GP map with the same genetic correlations can yield different levels of variation in correlated responses among selection lines. Some configurations could even give correlated responses in opposite direction to the prediction from selection and correlation (i.e. the Lande equation). The variation in the correlated response was higher for GP maps with more loci being modular than for more hidden pleiotropic GP maps. As there was no stabilizing selection on the second trait in Gromko's simulations, this was free to drift in the modular case but was constrained not to change (or change according to the level of genetic correlation) in the hidden pleiotropic case. In the present study, the situation is quite different as the second trait was under stabilizing selection. What I found when using the GP maps from Gromko's study was

that the response in trait 1 was significantly higher in the partially modular case (GP map with some pleiotropic loci generating correlations and some modular loci) than in the partially hidden pleiotropic case (GP map with pleiotropic loci that only partially cancel each other, thus generating correlations). This adds to Gromko's point that these GP maps are not equivalent although they give the same initial G matrix. Thus, even if no significant difference was found between the evolvability generated by a modular and a hidden pleiotropic GP map in the case of uncorrelated characters (section 4.1.1 and 4.1.3), this is not necessarily the case when genetic correlation are present, the modular structure having higher flexibility to counteract the unwanted correlated responses when opposing selective forces are present. This phenomenon seems only evident for the selection limit, however, as a small difference was noted in the short term response also in the no correlation scenario (section 4.1.1 and 4.1.3).

The main result form the hidden pleiotropy experiment, that hidden pleiotropy does not impose constraint on evolvability, seems to be in partial disagreement with a study by Baatz and Wagner (1997). They found that the rate of evolution of the focal character is reduced by a term proportional to $Cov(x_1, x_2^2)$ (cf. section 3.2.4). This effect is indeed repeated in this study (section 4.1.5) where the biallelic system yielded a significant constraint of hidden pleiotropy compared to modularity. In their stochastic model Baatz and Wagner (1997) included mutation. Those simulations yielded a lower evolutionary response with hidden pleiotropy than the theoretical prediction without any constraining pleiotropy. The degree of constraint was dependent on the strength of directional and stabilizing selection. A similar result was found by Griswold (2006). He investigated by simulations whether modularity increases evolvability (i.e. whether pleiotropy reduces evolvability). He introduced mutations with varying level of pleiotropy (n = the number of traits affected by mutation) whose effects on the various traits could be of different size and sign, and be correlated in varying degree and sign. Selection on a trait could be either directional or stabilizing. When only considering directional selection on all traits, he found that increasing the level of pleiotropy reduced evolvability when the average mutational correlation was negative and the average mutational effect per trait was zero, as well as when the average mutational correlation was zero and the average mutational effect was negative. Increasing the level of pleiotropy increased evolvability when the average mutational correlation was positive and the average mutational effect was zero, as well as when the average mutational correlation was zero and the average mutational effect was positive. When both the average mutational effect and the pleiotropic

effects of mutations were uncorrelated, increasing the level of pleiotropy did not affect adaptation. He also found that evolvability of a trait under directional selection decreases when it is pleiotropically associated with a trait under stabilizing selection, but increasing n for directionally selected traits relative to traits under stabilizing selection increased evolvability per trait for traits under directional selection. Griswold used an average mutational correlation of zero when considering sets of traits both under directional and stabilizing selection, thus corresponding to hidden pleiotropy. The results of the simulations where additive genetic variance was generated only through mutations (section 4.1.6) are in agreement with Baatz and Wagner (1997) and Griswold (2006) with regard to whether hidden pleiotropy constrains evolution under the current selective scenario. As a very small effect of linkage disequilibrium on the selection limit was found (in section 4.1.9), the hypothesis that these effects are not important for the current experiments is supported. However, as small differences in the responses between some of the GP maps are seen, it is reasonable to control for such potential effects. When modularity implies the total lack of gene effects on other traits than the focal one, the risk of interpreting constraints due to LD as effects of pleiotropy is present. This could be the case in the mutation-based experiment (section 3.2.5) where I used a modular GP map that excluded LD effects to conform to the system of Griswold (2006). However, these results were only meant to be qualitative as this experiment was not the main focus of the study.

As the answer to whether hidden pleiotropy reduces evolvability compared to modularity depends on the level of stabilizing selection, it should be noted that the value of s_2 used in my experiments (0.02) was strong enough to significantly reduce evolvability of the femur (trait 1), when there was stabilizing selection on humerus (trait 2) for the estimated mouse B matrix. Compared to that matrix the hidden pleiotropic matrix was non-constraining. The use of strong stabilizing selection (e.g. $s_2 = 0.68$) in mutation-based studies with moderate mutation rates is not directly transferable to simulations with only standing genetic variance with $std = mstd$ (allelic standard deviation equaling the standard deviation of mutational effects), because the level of additive genetic variance in those cases would initially be much higher than in the experiments with mutation.

The above-mentioned results, however, only apply when initial correlations between the two traits are zero. A point that might have been overlooked in this discussion is that when there in fact are genetic correlations, and pleiotropy is only partially hidden, a more modular GP

map, which gives the same G matrix as the pleiotropic one, yields significantly higher evolvability (section 3.2.7 and 4.1.8). This is especially the case for the selection limit. When there is an asymmetry between loci with synergistic and antagonistic pleiotropic effects, variation at available modular loci can counteract the correlated responses and thus enhance evolvability. Whether or not such a variational source is present is not evident from the G matrix alone. The experiment with the *even B matrices* also demonstrates that the structure of the GP map is important although the G matrix is unaffected, as these matrices yielded lower responses than the comparable modular and hidden pleiotropic ones, at least after some generations. They were in this respect also internally not 100% equal. When it comes to the selection limit, this experiment and the experiment where I compared a *regular* with an *irregular* hidden pleiotropic B matrix (section 4.1.7), provide evidence for the hypothesis that evolvability is governed by the absolute lengths of the projections of the allelic effects onto the axis of the selection gradient, as well as by the structure of the G matrix according to the Lande equation. Pavlicev and Hansen (2011) studied GP maps that maximize average evolvability (both character evolvability and evolvability in any arbitrary direction in phenotype space). They investigated different GP maps under the assumptions of the *character model* and the *trait model*. In the character model the addition of a pleiotropic effect of a locus on a trait does not change the effect on an already affected trait (This is an important assumption e.g. in the experiment of section 3.2.10.). The trait model however, assumes a constant effect size of each locus regardless of how many traits it affects (see also section 2.2), or more precisely the effect of each locus is a vector of constant length in phenotype space. In that case, Pavlicev and Hansen found that any GP map avoiding correlations maximizes evolvability (whether hidden pleiotropic or modular), as any such structures yield the same G matrix. However, if we only take account of standing genetic variation, as I do for most of the part in this study, the conclusions reached here imply that if a pleiotropic GP map can increase the number of underlying loci affecting each trait compared to a modular GP map, the selection limit is only maximized with the hidden pleiotropic map (cf. section 4.1.7). Another point worth mentioning, which also speaks in favor of the hidden pleiotropic structure as an optimal GP map, is that it is economical with regard to the number of loci needed. Given the assumptions I have made in my experiments, it is evident from the B matrices that the modular GP maps require more loci than the hidden pleiotropic ones (cf. e.g. section 3.2.1) when both traits are to be accounted for. As the genome necessarily must have a limited number of loci, a hidden pleiotropic structure might be more realistic than a

modular one (Walsh and Blows 2009). In conclusion, my results provide evidence for the claim that modularity is not necessary for evolvability (Hansen 2003). For further research it would be interesting to test how this conclusion is affected by the number of traits. It could be that hidden pleiotropy still would not constrain evolution, but as the modular GP map tended to yield slightly higher responses, there could be an underlying constraining effect of pleiotropy that could be reinforced when more traits are included. If so, it would be interesting to see how this may relate to Fisher's geometrical model (Fisher 1930), (cf. section 2.2).

The fact that pleiotropy can potentially be advantageous for evolvability has been shown earlier (Cheverud 1984; Hansen 2003). An attempt at quantifying this advantage is made in this study. Under certain assumptions about how more pleiotropic genomes differ from less pleiotropic ones (section 3.2.10) it is shown that in terms of the selection limit, an intermediate level (around 1/3 of the loci) of pleiotropy is significantly increasing the evolvability. Hansen (2003) predicted highest evolvability when 16% of the variation is pleiotropic. This corresponds to 16% pleiotropy in my experiment. For the selection limit the optimal level of pleiotropy found was 36%. In that case the total response was 24% higher than in the modular case. It was also shown that an intermediate level of pleiotropy was beneficial also in the short term response, and that within the very few first generations, even a higher level of pleiotropy than 36% could be the optimal GP map. The predictions of Hansen (2003) however, applies to the rate of evolution after an equilibrium between the different selective forces has been reached (i.e., after trait 2 has stopped responding), as it is based on the *conditional evolvability*. When analyzing the rates after this equilibrium the trend is still that evolvability as a function of pleiotropy increases in the beginning before it drops to almost zero, but the results exhibit much noise. However, the single highest value was indeed at 16% pleiotropy, a result which thus supports Hansen's prediction.

As mentioned earlier, there were effects of stabilizing selection on the relationship between the evolvability of the modular and the hidden pleiotropic GP maps. The stronger the stabilizing selection, the larger the difference in favor of the modular map. Over the total range of different strengths of stabilizing selection that I used, this effect was not very large for either evolutionary time span (around 10%). A rather surprising result, however, was that the short-term evolvability increased with increasing strength of stabilizing selection. This was found for both the modular and for the hidden pleiotropic GP map. The effect was

strongest for the modular GP map. This was not the case for the selection limit. For the fully constrained GP map (100% genetically correlated traits) the response was reduced in an approximately linear manner as stabilizing selection got stronger. By the same explanation as for why stronger stabilizing selection would reduce evolvability under the assumption of hidden pleiotropy in the mutation-based case (section 4.1.6), I would expect to observe a reduction in selection response as stabilizing selection got stronger here as well. This would not apply for the modular GP map, as the underlying genetic bases of the two traits are completely independent (disregarding LD). Since the unexpected positive effect of stabilizing selection was stronger for the modular GP map, a reduction in evolvability for the pleiotropic map could still be present, but with another larger effect working the opposite way that would mask it. One possibility is that a reduction in effective population size could be the cause. As stabilizing selection increases, the expected initial fitness decreases according to $E[W] = 1 - s_2 \cdot (n \cdot 2 \cdot std^2 + 1)$ (section 3.1.2). However, none of the values of s_2 that I have used in this experiment would yield $E[W] < 0$, so the potential effect should not be very large. In addition, this would be expected to reduce the response rather than to increase it in accordance with the results of reduced population size (section 4.1.3).

Hansen and Houle (2008) have developed measures of evolvability that are able to give predictions of the evolution of multiple traits that share additive genetic variance. They suggest that the conditional evolvability of Hansen (2003), derived to predict the short term evolution based on standing genetic variance, can be useful for making macroevolutionary predictions, as it may provide reliable information about the underlying constraints of the genetic architecture. I have tested how well conditional evolvability can predict evolution over several generations under different GP maps, and thus how good a measure it is of evolvability. The results showed that this measure gave good predictions across the whole time span considered (about 200 generations) for both a GP map of 36% pleiotropy and one with 68% pleiotropy, both when all effects were synergistic and when they were antagonistic. When the level of pleiotropy was high (68%) predictions from the Lande equation when trait 2 was disregarded gave predictions far away from reality, and even when both traits were included in the Lande equation (the selection gradient then having to be approximated) its predictions after some generations were not as good as those of the conditional evolvability. This measure is, however, also using the Lande equation, but it has the advantage of simplifying it by only considering one trait and its conditional variance. It thus seems that this variance is a good measure of evolvability regardless of the underlying genetic architecture.

However, more GP maps should be tested, including hidden and partially hidden pleiotropic B matrices, and also different allelic distributions should be considered.

5.1   Conclusions

Many important aspects of the genotype-phenotype map are not simply reflected by the genetic variance-covariance matrix (G). Features that may significantly affect evolvability include the number of loci and the pleiotropy structure in many regards. Although modularity emerges immediately as a strong candidate for optimizing evolvability, and it has here been demonstrated that this might in several scenarios be true, there are also several arguments that speak in favor of a pleiotropic GP map as the optimal genetic architecture. It has been shown that even over many generations of selection, hidden pleiotropy does not necessarily produce any constraints on evolvability, and in some respects pleiotropy may also enhance evolvability. Still, much is derivable from G alone. *Conditional evolvability* seems to be a good measure of evolvability, which is robust with respect to the GP map. It should be tested with other genetic architectures than has been done here, and for multiple traits, in order to evaluate its general applicability. The same should be done for the other experiments in this study, as it is unrealistic that the number of genetically associated traits in natural organisms would be limited to two. Further, before these and other theoretical results can be reliably generalized for the real world, more knowledge is needed about the genetic and selective conditions in nature. However, this thesis may have helped illuminate some aspects of how genetic architecture can affect the evolution of quantitative traits.

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Appendix

The script

The simulation is run by opening the RGui, and clicking on "Source R code" (menu "File")
and choosing the file under which the below script has been saved. A short description
appears and the program prompts the user for parameter values. The following parameters
must be provided: the number of recursions (c), the number of generations (t) and the
population size (N). Next, the program presents the fitness function and asks for the selection
coefficients (s_1 and s_2), the allelic mean (m), standard deviation (std) and the environmental
standard deviation ($estd$). A selection of possible B-matrices appears, from which one should
be chosen by entering a corresponding number. Alternatively, one may enter "no" to create a
new B-matrix. In that case the program prompts the user for the entries in the B-matrix one by
one row-wise. Each entry is written as a number and submitted by pressing enter. When a row
is finished, press enter once more without writing anything. The rows must be of equal length.
When the B-matrix (B) is entered the simulation starts. By entering the variables as
commands in the Rgui console, R returns their values. One can limit the output to a desired
subset by indexes (in the brackets), either by a single number or a vector. An empty index
gives the whole set of values in the respective dimension.

The following script does not include all the B matrices used in the experiments, nor the code for the other distributions of allelic effects than the normal distribution and also not the code for the mutation-based simulations.

```
# The Evolution of Two Traits: z2 and z2
cat("The Evolution of Two Traits: z1 and z2\n") ;
cat("THE MODEL: (z1,z2) = start-values + B-matrix * y-vector + environmental variance-vector\n") ;

# THE MODEL: (z1,z2) = start-values + B-matrix * y-vector + environmental variance-vector
#  -->      (z1,z2) = sv + B * y + e

# Parameters
cat("\n") ;
cat("Please set parameter values to run program.\n") ; cat("\n") ;
c <- readline(" number of recursions (c) = ") ;
c <- as.double(c) ;
t <- readline(" number of generations (t) = ") ;         # generations
t <- as.double(t) ;
N <- readline(" population size (N) = ") ;            # individuals
N <- as.double(N) ;
L <- 20                           # loci
P <- 2                            # ploidity      (fixed)
cat("fitness function: W = 1 + s1*(z1-mean(z1)) - s2*(z2-optz2)^2 \n") ;
s1 <- readline(" selection coefficient 1 (s1) (default: 0) = ") ;     # strength of selection on z1
if (s1=="") s1 <- 0 ;
s1 <- as.double(s1) ;

s2 <- readline(" selection coefficient 2 (s2) (default: 0) = ") ;     # strength of selection on z2
if (s2=="") s2 <- 0 ;
s2 <- as.double(s2) ;

# Parametres in initial population (normally distributed allelic values):

m <- readline(" allelic mean value (m) (default: 0) = ") ;  # mean  allelic value
if (m=="") m <- 0 ;
m <- as.double(m) ;

std <- readline(" allelic standard deviation (std) (default: 0.5) = ") ;  # standard deviation of allelic value
if (std=="") std <- 0.5 ;
std <- as.double(std) ;

estd <- readline(" environmental standard deviation (estd) (default: 1) = ") ;  # environmental standard deviation
if (estd=="") estd <- 1 ;
estd <- as.double(estd) ;

# "Start-vector" (neutral values of the X's without the allelic effects)

sv <- c(10,10)

cat("\n") ;
cat("Please choose one of the following B-matrices by entering the corresponding number (1-11) or enter << no
>>
to write your own.\n") ; cat("\n") ;

# Define B-matrix (must be a (2 x L)-matrix):

bb11 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
```

```
bb12 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb1 <- rbind(bb11,bb12)

bb21 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb22 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,-1)
bb2 <- rbind(bb21,bb22)

bb31 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb32 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,-1,-1)
bb3 <- rbind(bb31,bb32)

bb41 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb42 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,-1,-1,-1)
bb4 <- rbind(bb41,bb42)

bb51 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb52 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,-1,-1,-1,-1)
bb5 <- rbind(bb51,bb52)

bb61 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb62 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,-1,-1,-1,-1,-1)
bb6 <- rbind(bb61,bb62)

bb71 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb72 <- c(1,1,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0,0,0,0)
bb7 <- rbind(bb71,bb72)

bb81 <- c(0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1)
bb82 <- c(0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
bb8 <- rbind(bb81,bb82)

bb91 <- c(1.494537,2.769312,0.0007735036,-0.04667998,-0.006092008,-0.02388131,-
0.2719293,0,0,0.1433124)    # mouse matrix
bb92 <- c(0.764224,2.998482,0.3087173697,0.07636481,0.001893725,-0.01275512,-0.2862922,0,0,-0.0513877)
bb9 <- rbind(bb91,bb92)

bb101 <- c(1.414214,1.144123,0.437016,-0.437016,-1.441228,-1.414214,-1.144123,-
0.437016,0.437016,1.144123)     # even B matrix
bb102 <- c(0,0.8312539,1.344997,1.344997,0.8312539,1.731855e-16,-0.8312539,-1.344997,-1.344997,-
0.8312539)
bb010 <- rbind(bb101,bb102)

bb111 <- c(0,0.8312539,1.344997,1.344997,0.8312539,1.731855e-16,-0.8312539,-1.344997,-1.344997,-
0.8312539)   # even B reversed
bb112 <- c(1.414214,1.144123,0.437016,-0.437016,-1.441228,-1.414214,-1.144123,-
0.437016,0.437016,1.144123)
bb011 <- rbind(bb111,bb112)

cat("(1) ");cat(bb11);cat("\n");cat("    ");cat(bb12);cat("\n");cat("\n") ;
cat("(2) ");cat(bb21);cat("\n");cat("    ");cat(bb22);cat("\n");cat("\n") ;
cat("(3) ");cat(bb31);cat("\n");cat("    ");cat(bb32);cat("\n");cat("\n") ;
cat("(4) ");cat(bb41);cat("\n");cat("    ");cat(bb42);cat("\n");cat("\n") ;
cat("(5) ");cat(bb51);cat("\n");cat("    ");cat(bb52);cat("\n");cat("\n") ;
cat("(6) ");cat(bb61);cat("\n");cat("    ");cat(bb62);cat("\n");cat("\n") ;
cat("(7) ");cat(bb71);cat("\n");cat("    ");cat(bb72);cat("\n");cat("\n") ;
cat("(8) ");cat(bb81);cat("\n");cat("    ");cat(bb82);cat("\n");cat("\n") ;
cat("(9) ");cat(bb91);cat("\n");cat("    ");cat(bb92);cat("\n");cat("\n") ;
cat("(10) ");cat(bb101);cat("\n");cat("     ");cat(bb102);cat("\n");cat("\n") ;
cat("(11) ");cat(bb111);cat("\n");cat("     ");cat(bb112);cat("\n");cat("\n") ;
cat("\n");
```

```
AN <- readline(" Choose a B-matrix (1-11) or type << no >> to set a new one: ") ;
cat("\n");

{if (AN == 1) B <- bb1 else if (AN == 2) B <- bb2 else if (AN == 3) B <- bb3 else if (AN == 4) B <- bb4
 else if (AN == 5) B <- bb5 else if (AN == 6) B <- bb6 else if (AN == 7) B <- bb7 else if (AN == 8) B <- bb8
else if (AN == 9) B <- bb9
 else if (AN == 10) B <- bb010 else if (AN == 11) B <- bb011
  else {cat("Enter the elements of the first row. Press enter key when done.\n"); bbb1 <- scan("");
     L <- length(bbb1);
     while (L < 2) { cat("At least two entrees please!\nEnter the elements of the first row.\n"); bbb1 <- scan("");
L <- length(bbb1); };
     cat("Enter the elements of the second row.\n"); bbb2 <- scan("",nmax=L);
     B <- rbind(rep(0,L),rep(0,L)); B[1,] <- bbb1 ; B[2,] <- bbb2 ;};} ;

L <- length(B[1,]) ;          # correction for when the predefined B matrices have unequal lengths
# L must be at least 2!!!

B1 <- B[1,] ; B2 <- B[2,] ;

cat("\n") ;
cat("B-matrix:\n") ;
print(B) ;

# Define fitness-function:

fW <- function (z1,z2,optz2,mz1) {

  1 + s1*(z1-mz1) - s2*(z2-optz2)^2    # Fitness ( z1 = trait one , z2 = trait two, optz2 = optimal value of trait
two, mz1 = mean value of trait one )

}

nw <- 0    # count of negative fitness-individuals
nnw <- 0   #  -"- after correction            (must be zero)
X1 <- rep(0,t) # mean of mean phenotypes
X2 <- rep(0,t)
XX1 <- seq(c*t)
dim(XX1) <- c(c,t)
XX2 <- seq(c*t)
dim(XX2) <- c(c,t)

RMW <- 0  # mean relative fitness increase
RRMW <- rep(0,c)

mG <- seq(2*2*t)    # mean G matrix
dim(mG) <- c(2,2,t)
G <- seq(2*2*t*c)   # G matrix
dim(G) <- c(2,2,t,c)
nmG <- seq(2*2*t)    # mean genetic correlation matrix
dim(nmG) <- c(2,2,t)
nG <- seq(2*2*t*c)     # genetic correlation matrix
dim(nG) <- c(2,2,t,c)

for (r in 1:c) {

# make an initial population by establishing a L*P*N-matrix (alleles)
# mean = m standard deviation = std

y2 <- rnorm(L*P*N,m,std) ;
```

72

dim(y2) <- c(L,P,N) ;

Other Variables/Functions:

make y-vectors for the individuals ((Z1,Z2) = sv + B-matrix * y-vector + e)
where y is the sum of the allelic values at each locus:

y3 <- c(seq(L*P*N*(t+1))) ; # matrix of allelic values, Establish a dimension for generation.
dim(y3) <- c(L,P,N,t+1) ;
y3[,,,1] <- y2 ;
y <- y3[,1,,] ; # This is now a L*N*c-matrix

e <- rnorm(2*N*t,0,estd) ; # Environmental component of phenotype
dim(e) <- c(2,N,t) ;

x <- seq(2*N*t) ;   # Genetic component of phenotype
dim(x) <- c(2,N,t) ;

g <- seq(2*2*t) ;   # preliminary G-matrix
dim(g) <- c(2,2,t) ;
xx <- seq(N*2*t) ;
dim(xx) <- c(N,2,t) ;

ng <- g ;   # preliminary genetic correlation matrix

xx1 <- seq(N*t) ; # Partition x in two variables (one per trait)
dim(xx1) <- c(N,t) ;
xx2 <- seq(N*t) ;
dim(xx2) <- c(N,t) ;

# mean phenotypes:
x1 <- seq(t) ;
x2 <- seq(t) ;

# optimal trait2-value (for stabilizing selection)
optz2 <- NA ;

W <- c(seq(N*t)) ;   # fitness
dim(W) <- c(N,t) ;

# Original fitness before negatives are set to zero, for statistical purposes tW(W):
tW <- seq(N*t) ;
dim(tW) <- c(N,t) ;

mW <- c(seq(t)) ; # mean population fitness

w <- c(seq(N*t)) ; # relative fitness
dim(w) <- c(N,t) ;

cumint <- c(seq(N*t)) ;   # cumulative intervals
dim(cumint) <- c(N,t) ;

scumint <- c(seq((1+N+1)*t)) ; # scaled cumulative intervals
dim(scumint) <- c(1+N+1,t) ;

selm <- runif(N*P*t) ; # selection matrix
dim(selm) <- c(N,P,t) ;

selind <- N*P*t + 1 + c(seq(N*P*t)) ; # list of selected individuals
dim(selind) <- c(N,P,t) ;

```
bip <- seq(L*P*P*N*t) ;      # reproduction matrix
dim(bip) <- c(L*N,P,P,t) ;

y4 <- seq(L*N*P*t) ;         # preliminary allele matrix
dim(y4) <- c(L*N,P,t) ;

for (i in 1:t) {

for (j in 1:N) y[,j,i] = y3[,1,j,i] + y3[,2,j,i] ;  # summing alleles at each locus

    # y is a L*N*t-matrix

# phenotypes x(B,y):

for (j in 1:N)
   x[,j,i] = sv + B %*% y[,j,i] ;

xx1[,i] <- x[1,,i] ;
xx2[,i] <- x[2,,i] ;
xx[,1,i] <- xx1[,i] ;
xx[,2,i] <- xx2[,i] ;

g[,,i] <- cov(xx[,,i],xx[,,i])*((N-1)/N) ;      # G-matrix ("population variance")
for(aa in 1:2) for(ab in 1:2) {
ng[aa,ab,i] <- g[aa,ab,i] / (sqrt(g[aa,aa,i])*sqrt(g[ab,ab,i])) } ; # genetic correlation matrix

G[,,i,r] <- g[,,i] ; # for calc. of mean G-matrix (over c)
nG[,,i,r] <- ng[,,i] ; # "--" cor.

for (j in 1:N)
x[,j,i] = x[,j,i] + e[,j,i] ;
x1[i] <- mean(x[1,,i]) ;
x2[i] <- mean(x[2,,i]) ;

z1 <- x[1,,i] ;  # trait one
z2 <- x[2,,i] ;  # trait two

mz1 <- x1[i] ;  # mean of trait one
mz2 <- x2[i] ;  # mean of trait two  (not used with the current fitness function)

optz2 <- mean(xx2[,1]) ;  # optimal z2 (initial mean z2 without e)

# Fitness function W(z1,z2,optz2,mz1):

for(j in 1:N)
                W[j,i] = fW(z1[j],z2[j],optz2,mz1)  ;


# Original fitness before negatives are set to zero, for statistical purposes tW(W):
tW[,i] <- W[,i] ;

# Count negative fitness individuals:  nw(W) & nnw(W)

for(j in 1:N) if(W[j,i] < 0) { nw = nw + 1 ; W[j,i] = 0
 } ;
for(j in 1:N) if(W[j,i] < 0)  nnw = nnw + 1 ;

# Mean population fitness:  mW(W)
```

74

```
   mW[i] = mean(W[,i])   ;

if(mW[i]==0) { cat("\n"); cat("ERROR!!! Mean fitness = 0 in generation "); cat(i);
          cat(" in recursion "); cat(r); cat(".\n");
          cat("Please adjust parameter settings to avoid negative fitness values.\n"); cat("\n");
} ;

# Relative fitness:  w(W,mW)

for (j in 1:N)
          w[j,i] = W[j,i] / mW[i] ;

# Vector with cumulative intervals of relative fitnesses:    cumint(w)

for (j in 1:N)  cumint[j,i] = sum(w[1:j,i]) ;

# Vector with scaled cum.int. to sum up to 1:     scumint(cumint,N)

scumint[1,i] <- 0 ;
scumint[1+N+1,i] <- 2;
for (j in 1:N)
          scumint[1+j,i] = cumint[j,i] / N  ;

# Selection:
# selection matrix (N*P*t):   selm(N,P,t)

# make list of selected individuals:  selind(scumint,selm)

for (p in 1:P)
   for (j in 1:N) {
          a <- 2 + floor(selm[j,p,i] * (N-1)) ;
          k <- 0 ;
          while(1 != k)
           if (selm[j,p,i] > scumint[a,i]) { if(selm[j,p,i] <= scumint[a+1,i]) { selind[j,p,i] = a ; k = 1 } else a = a
+ 1
             } else if(selm[j,p,i] > scumint[a-1,i]) { selind[j,p,i] = a-1 ; k = 1 } else a = a-1}  ;

#Reproduction:

# Random binominal
nn <- N*P*L ;

gt <- runif(nn)*10 ;
bik <- gt%/%5 ;
bil <- (-1)*(bik-1) ;
bim <- cbind(bik,bil) ;
dim(bim) <- c(length(bik)/P,P,P) ;
bim[,1,1] <- bik[1:(length(bik)/2)] ;
bim[,2,1] <- bil[1:(length(bil)/2)] ;
bim[,1,2] <- bik[(length(bik)/2+1):length(bik)] ;
bim[,2,2] <- bil[(length(bil)/2+1):length(bil)] ;


for (j in 1:N) { bip[((j-1)*L+1):(j*L),1,1,i] = y3[,1,selind[j,1,i],i] ;  bip[((j-1)*L+1):(j*L),2,1,i] =
y3[,2,selind[j,1,i],i] ;
          bip[((j-1)*L+1):(j*L),1,2,i] = y3[,1,selind[j,2,i],i] ;  bip[((j-1)*L+1):(j*L),2,2,i] = y3[,2,selind[j,2,i],i]
;
          } ;

y4[,1,i] <- bip[,1,1,i]*bim[,1,1] + bip[,2,1,i] * bim[,2,1] ;
```

```r
y4[,2,i] <- bip[,1,2,i]*bim[,1,2] + bip[,2,2,i] * bim[,2,2] ;

for (j in 1:N)
   for(p in 1:P)  y3[,p,j,i+1] = y4[((j-1)*L+1):(j*L),p,i] ;

XX1[r,i] <- x1[i] ;  # for calc. of mean of mean phenotype
XX2[r,i] <- x2[i] ;

} ;  # end of i-loop

rmw <- (mW[t] - mW[1]) / mW[1] ;   # relative augmentation in mean fitness through the whole time span
RRMW[r] <- rmw ; # for calc. of mean of mean fitn. incr.

# plot phenotypes against generation (x1-black, x2-blue)
plot(seq(t),x1,ylim=c(min(x1,x2)-0.1*sqrt((min(x1,x2))**2),max(x1,x2)+0.1*sqrt((max(x1,x2))**2)),"l",
  xlab="Generation",ylab="Mean Phenotype") ;
lines(seq(t),x2,col="blue") ;
text(0.1*t+1,max(x1,x2),r) ;
text(0.15*t+1,max(x1,x2)," of ") ;
text(0.20*t+1,max(x1,x2),c) ;

} # end of r-loop

for (i in 1:t) {
X1[i] <- mean(XX1[,i]) ;  # mean of mean phenotypes
X2[i] <- mean(XX2[,i]) ;

# mean G-matrix and mean genetic correlation matrix
mG[1,1,i] <- mean(G[1,1,i,]) ;
mG[1,2,i] <- mean(G[1,2,i,]) ; # !!! remember these are means of numbers that may have opposite sign!!!
mG[2,1,i] <- mean(G[2,1,i,]) ; # !!! remember these are means of numbers that may have opposite sign!!!
mG[2,2,i] <- mean(G[2,2,i,]) ;
nmG[1,1,i] <- mean(nG[1,1,i,]) ;
nmG[1,2,i] <- mean(nG[1,2,i,]) ; # !!! remember these are means of numbers that may have opposite sign!!!
nmG[2,1,i] <- mean(nG[2,1,i,]) ; # !!! remember these are means of numbers that may have opposite sign!!!
nmG[2,2,i] <- mean(nG[2,2,i,]) ;

}

RMW <- mean(RRMW)      # mean of mean fitness increase

# plot (mean of mean) phenotypes against generation (X1-black, X2-blue)
plot(seq(t),X1,ylim=c(min(X1,X2)-0.1*sqrt((min(X1,X2))**2),max(X1,X2)+0.1*sqrt((max(X1,X2))**2)),"l",
  xlab="Generation",ylab="Mean Phenotype")
lines(seq(t),X2,col="blue")
legend(1,max(X1,X2),c("trait 1","trait 2"),fill=c("black","blue"))

# print parameter values
cat("\n") ; cat("\n") ;
cat("Input \n");
cat("c = ");cat(c);cat("\n") ;
cat("t = ");cat(t);cat("\n") ;
cat("N = ");cat(N);cat("\n") ;
cat("s1 = ");cat(s1);cat("\n") ;
cat("s2 = ");cat(s2);cat("\n") ;
cat("m = ");cat(m);cat("\n") ;
cat("std = ");cat(std);cat("\n") ;
cat("estd = ");cat(estd);cat("\n") ;
cat("\n") ;
cat("B = ");cat(B1);cat("\n");cat("    ");cat(B2);cat("\n") ;
```

```
# print results
cat("\n") ;
cat("Output \n");
cat("G[,,1,1] = \n");print(G[,,1,1]);cat("\n") ;
cat("G[,,t,1] = \n");print(G[,,t,1]);cat("\n") ;
cat("mG[,,1] = \n");print(mG[,,1]);cat("\n") ;
cat("mG[,,t] = \n");print(mG[,,t]);cat("\n") ;
cat("X1[t] - X1[1] = ");cat(X1[t]-X1[1]);cat("\n") ;
cat("X2[t] - X2[1] = ");cat(X2[t]-X2[1]);cat("\n") ;

# for the plotting of (cumulative) evolvabilities against generations

gru <- seq(t-1)
for(fat in 2:t) gru[fat-1] = (X1[fat]-X1[1])/(fat-1)

# for statistics:
gruu<- seq((t-1)*c);
dim(gruu)<-c((t-1),c);
for(fat in 2:t) gruu[fat-1,]=(XX1[,fat]-XX1[,1])/(fat-1);
```