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RESEARCH ARTICLE

Genotype-Phenotype Maps Maximizing Evolvability: Modularity Revisited

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Abstract The mechanisms translating genetic to phenotypic variation determine the distribution of heritable phenotypic variance available to selection. Pleiotropy is an aspect of this structure that limits independent variation of characters. Modularization of pleiotropy has been suggested to promote evolvability by restricting genetic covariance among unrelated characters and reducing constraints due to correlated response. However, modularity may also reduce total genetic variation of characters. We study the properties of genotype-phenotype maps that maximize average conditional evolvability, measured as the amount of unconstrained genetic variation in random directions of phenotypic space. In general, maximal evolvability occurs by maximizing genetic variance and minimizing genetic covariance. This does not necessarily require modularity, only patterns of pleiotropy that cancel on average. The detailed structure of the most evolvable genotype-phenotype maps depends on the distribution of molecular variance. When molecular variance is determined by mutation-selection equilibrium either highly pleiotropic or highly modular genotype-phenotype maps can be optimal, depending on the mutation rate and the relative strengths of stabilizing selection on the characters.

Keywords Pleiotropy · Constraint · Correlated response · Genetic architecture · Conditional evolvability

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Introduction

Evolvability is the ability to respond to a selective challenge by a genetically-based phenotypic change. The ability of a character to evolve depends on its amount of heritable phenotypic variance, and on its genetic covariance with other characters, as summarized in the genetic variance matrix, **G** (Lande 1979). Underlying molecular genetic variation is mapped onto phenotypic variation by a complex of processes commonly referred to as the genotype-phenotype (GP) map. In particular, genetic covariances are (mainly) caused by pleiotropy in the genotype-phenotype map (Lande 1980). Such covariance may constrain the ability of characters to respond to selection if their covarying counterparts are exposed to conflicting selection regimes.

Because evolution requires a degree of quasi independence among characters (Lewontin 1978), pleiotropy is a potential cause of evolutionary constraints. The rationale is that the more traits are affected by a mutation, the harder it becomes to adapt, because a random mutation is less likely advantageous if it affects many traits simultaneously (Fisher 1958; but see Kimura 1983; Waxman and Welch 2005). This has led to suggestions that the genotype-phenotype maps that optimize evolvability are those where pleiotropy is restricted to sets of traits with related function or development for which the orchestrated responses are advantageous (Olson and Miller 1958; Berg 1960; Riedl 1978; Cheverud 1984, 1996; Wagner 1996; Wagner and Altenberg 1996). The advantage of modular genotype-phenotype maps is twofold. First, if pleiotropic effects of most genes are restricted to few traits, the risk of deleterious effects of a mutation is reduced (modularity). Second, if the traits affected by a gene are developmentally or functionally related, a mutation may preserve functionality of this integrated module during evolutionary change

(integration). Based on the premise that selection can mould the genotype-phenotype map to increase evolvability, this idea prompted studies to assess the modularity of genetic effects and patterns of variation (reviewed in Wagner et al. 2007; Mitteroecker 2009). It is still controversial, however, whether genotype-phenotype maps can be regarded as adaptations for evolvability (see, e.g., Hansen 2006; Jones et al. 2007; Lynch 2007a, b, c; Draghi and Wagner 2008; Pavlicev et al. 2008, 2011a; Hansen 2011, for a variety of opinions). Regardless of the question of its evolution, it is not obvious that a modular genotype-phenotype map maximizes evolvability. In contrast to the intuitive advantages mentioned above, Hansen (2003) pointed out that modularity in the form of reduced pleiotropy might also hamper evolvability by reducing the potential for genetic variance per trait (see also Chevin et al. 2010). Below a certain level of pleiotropy, the variational independence gained does not compensate for the genetic variability lost due to smaller genetic basis. Hansen (2003) explored this effect in various genotype-phenotype maps and suggested that modular maps may not generally maximize trait evolvability. Instead, the evolvability generated by a genotype-phenotype map may depend on several factors, such as the sign of genetic effects, the strength of stabilizing selection at the mutation-selection equilibrium, and the distance from the fitness optimum. He suggested that full, but maximally variable, pleiotropy may optimize evolvability. Here, we explore this idea in more detail.

Pleiotropy generates genetic correlations only if the shared effects on traits are biased towards the same effects, e.g., if single loci systematically generate either positive or negative covariances that therefore do not cancel out across loci. Thus, while nonzero genetic correlation is usually a sign of pleiotropy, the lack of genetic correlation does not require a lack of pleiotropy. Multiple ways to generate similar correlation structures have been discussed before (e.g., Cheverud 1984; Wagner 1989; Charlesworth 1990; Houle 1991; Gromko 1995; Hallgrimsson et al. 2009; Mitteroecker 2009; Roseman et al. 2009). In this paper, we use Wagner's (1989) **B**-matrix model in a two-trait system to systematically describe which patterns of pleiotropy maximize conditional evolvability in the sense of Hansen and Houle (2008). We explore several different assumptions about the maintenance of genetic variation on underlying loci. Our results confirm that the absence of pleiotropy is not the only way of maintaining high evolvability, but if there are no constraints on the type of pleiotropy that can occur, evolvability is generally maximized by maps that tend to generate zero genetic correlation either through modularity, or through patterns of pleiotropy that cancel out. The detailed results depend, however, on the mechanisms shaping genetic variance.

Model

Modeling the Genotype-Phenotype Map

We represent the GP map by the **B**-matrix model of Wagner (1989), which maps "molecular" genetic variation in a vector of n underlying genes, $\mathbf{y}$, linearly into a vector of N phenotypic traits, $\mathbf{z}$, as $\mathbf{z} = \mathbf{B}\mathbf{y}$:

$$\begin{pmatrix} z_1 \\ . \\ z_N \end{pmatrix} = \begin{pmatrix} b_{11} & \ldots & b_{1n} \\ . & & . \\ b_{N1} & \ldots & b_{Nn} \end{pmatrix} \begin{pmatrix} y_1 \\ . \\ y_n \end{pmatrix}.$$

We will henceforth use the expressions **B** matrix and genotype-phenotype map interchangeably. The evolvability is measured on the genetic covariance matrix, **G**. We express the **G** matrix in terms of the **B** matrix elements applying the relationship $\mathbf{G} = \mathbf{B}\mathbf{V}\mathbf{B}^{\mathrm{T}}$, where **V** is a matrix of underlying gene-specific variances. We can think of **V** as representing molecular genetic variance on some unspecified scale. We then use measures of evolvability developed for the genetic covariance matrix **G** by Hansen and Houle (2008), and Hansen et al. (2003a). We assume linkage equilibrium, which implies that the **V** matrix is diagonal, and therefore that all trait covariance is due to pleiotropy. Three alternative assumptions about the structure of the **V** matrix are explored. In the first, we assume that all loci have the same level of molecular variance. In the second and third, we assume that the molecular variance is kept in balance between mutation and stabilizing selection on the traits according to the house-of-cards approximation, which is valid for rare mutations with large effects (Turelli 1984) or the Gaussian approximation, which is valid for frequent mutations of individually small effects (Lande 1980). We are aware of the existence of more general models of mutation-selection balance (Welch and Waxman 2002; Hermisson and Wagner 2004), but the two "extreme" cases will serve to illustrate the qualitative range of potential effects of pleiotropy on evolvability. In both models, stabilizing selection occurs according to the fitness function:

$$W(z_1, z_2) = k \ \exp\left[-\frac{1}{2}\left(s_1 z_1^2 + s_2 z_2^2\right)\right],$$

where k is a constant, and s_i is the strength of selection along the phenotypic axis of the character i. Thus, we assume no correlated selection on the two characters. In principle, correlated selection is accounted for in our framework by a transformation of the major axes of correlated selection to the character axes (Wagner 1989). Hence, the assumption of no correlated selection is equivalent to defining characters as the independent directions of selection. Note that $s_1 = s_2$ corresponds to uniform stabilizing selection in all directions in morphospace. The marginal fitness of the y_j values at each locus is then

$$W(y_j) = k\ \exp\left[-\frac{1}{2}y_j^2\left(s_1 b_{1j}^2 + s_2 b_{2j}^2\right)\right].$$

Based on this, Wagner (1989) showed that the equilibrium single-locus variances under the house-of-cards approximation are:

$$V_{y_j} \approx \frac{4u_j}{s_1 b_{1j}^2 + s_2 b_{2j}^2}, \tag{1}$$

where u_j is the mutation rate at the locus j. These equilibrium variances at single loci are the entries of the $n \times n$ diagonal matrix $\boldsymbol{V}$. The equilibrium single-locus variance under the Gaussian approximation is:

$$V_{y_j} \approx \sqrt{\frac{\sigma_{mj}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}}, \tag{2}$$

where $\sigma_{mj}^2 = u_j \gamma_j^2$ is the "molecular" mutational variance arising at locus j in the population per generation, u_j is the locus-specific mutation rate and γ_j^2 is the variance in effects of the new mutations at locus j on the underlying molecular scale. We will assume in the following that the mutational variance is uniform across loci and equal to σ_m^2. See also Lande (1980) and Turelli (1985) for basic results on mutation-selection balance in pleiotropic systems.

Measures of Evolvability

The evolvability of a trait is influenced by the amount of genetic variation and its entanglement with other traits. Several evolvability measures can be defined depending on different assumptions about selection and constraint due to correlated response. For derivation and details see Hansen and Houle (2008). Here we briefly list the measures relevant for this study.

The unconditional evolvability predicts the response to unit selection (i.e., a mean-scaled selection gradient of length one along the specific dimension in space (Hansen et al. 2003b)) and equals the amount of additive genetic variance in that direction:

$$e(\beta) = \beta^T \mathbf{G} \beta,$$

where β is a selection gradient normalized to unit length.

When addressing autonomization of traits, we assume that correlated response of other traits has negative effects on fitness. The conditional evolvability is defined as the response to selection along specific direction when this is in equilibrium with stabilizing selection in all orthogonal directions:

$$c(\beta) = \left(\beta^T \mathbf{G}^{-1} \beta\right)^{-1},$$

where β is a selection gradient normalized to unit length. This is independent of the strength of constraining selection, since constraining traits under weaker selection are simply pulled further from their optimum and at equilibrium they will exert the same constraining force as a trait under stronger selection (Hansen 2003).

The above are measures of evolvability in specific directions of morphospace, which could be along the trait axes or in any direction of correlational selection. These equations show that evolvabilities will typically be different in different directions. To obtain general measures of the evolvability inherent in a genetic system we therefore need to average over several possible directions. We do this in two different ways.

One is to take the average across the two character axes. This assumes that directional selection tends to act on the two characters separately and at different times. Hence, we take the average of the evolvability along the selection vectors $\beta_1 = \begin{pmatrix} 1 \\ 0 \end{pmatrix}$ and $\beta_2 = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$. We will use an asterisk to denote this average (character) evolvability.

The other way is to average evolvability across all possible directions in morphospace. This assumes that selection is equally likely on all combinations of the two traits. We will use a cross bar to denote this average across all directions.

Hansen and Houle (2008) showed that these averages are

$$\bar{e}^* = \frac{G_{11} + G_{22}}{2}, \tag{3}$$

$$\bar{e} = \frac{\lambda_1 + \lambda_2}{2} = \bar{e}^*, \tag{4}$$

$$\bar{c}^* = \frac{1}{2}\left(\frac{Det[\mathbf{G}]}{G_{11}} + \frac{Det[\mathbf{G}]}{G_{22}}\right), \tag{5}$$

$$\bar{c} = \sqrt{\lambda_1 \lambda_2} = \sqrt{Det[\mathbf{G}]}, \tag{6}$$

where G_{ii} is the genetic variance of trait i, G_{ik} is the genetic covariance between traits i and k, and λ_i are the eigenvalues of the genetic covariance matrix, $\mathbf{G}$.

The purpose of this work is to find the structures of the $\mathbf{B}$ matrix with highest evolvability. To this end, we can write the average evolvabilities as functions of the $\mathbf{B}$-matrix elements

$$\bar{e}^* = \bar{e} = \frac{1}{2}\sum_{j=1}^{n} V_j\left(b_{1j}^2 + b_{2j}^2\right), \tag{7}$$

$$\begin{aligned}\bar{c}^* &= \frac{1}{2} Det\left[\mathbf{B}\mathbf{V}\mathbf{B}^{\mathbf{T}}\right]\left(\left(\sum_{j=1}^{n} V_j b_{1j}^2\right)^{-1} + \left(\sum_{j=1}^{n} V_j b_{2j}^2\right)^{-1}\right) \\ &= \bar{e}^*\left(1 - \frac{\left(\sum_{j=1}^{n} V_j b_{1j} b_{2j}\right)^2}{\sum_{j=1}^{n} V_j b_{1j}^2 \sum_{j=1}^{n} V_j b_{2j}^2}\right),\end{aligned} \tag{8}$$

$$\bar{c} = \sqrt{Det[\mathbf{B}\mathbf{V}\mathbf{B}^{\mathbf{T}}]}, \tag{9}$$

where the sums are over all loci affecting the characters. These expressions are summarized for the particular assumptions about the underlying variance in Table 1.

Modeling Pleiotropy

In order to build realistic **B** matrices, we need to make an assumption about the relationship between the degree of pleiotropy and the magnitude of the effect per character. We use two models of pleiotropy: the *character model* and the *trait model.* The *character model* (also called Euclidian superimposition model in Wagner et al. 2008) assumes that the phenotypic measurements represent biologically meaningful directions in phenotypic space that we call characters (Wagner 2001). This implies that the genetic underpinning of the character is independent of the effects of its genes on other characters. The effect size of a gene *per given character* is thus modeled as constant, regardless of the number of other characters affected by the same gene (Fig. 1a). As a consequence, the total effect of a gene that affects multiple characters increases with the number of characters affected (see Salathe et al. 2006; Wagner et al. 2008; Wang et al. 2010; Wagner and Zhang 2011 for empirical support).

The alternative *trait model* (also named invariant total effect model in Wagner et al. 2008) assumes that phenotypic measurements, traits, are arbitrary directions in phenotypic space with no biological identity. We model this by assuming *a constant vector length of effects*, regardless of how many traits are affected. As a consequence, the genetic effect *per trait* decreases when the number of traits affected by a gene increases (Fig. 1b). Naively, this model predicts a reduction in evolvability per trait with increasing complexity, if pleiotropy is taken as a proxy for complexity (Orr 2000; Welch and Waxman 2003).

The two models delimit a continuum, as characters are hard to determine in practice and often do not coincide with measurements. Clearly, an arbitrary number of measurements can be taken on any part of the organism, which necessarily describe the same lower-dimensional structure. The total effect of an underlying gene in such redundantly assessed structure is limited, as some measurements are highly correlated and share the same genetic basis.

Results

The main results are summarized in Tables 2 and 3. We focus on conditional evolvabilities, as we are mainly interested in the constraining effects of conflicting

Table 1 A summary of expressions for calculating evolvabilities under different assumptions about variance distribution at underlying loci

Optimized criterion	Uniform variances at loci	Variances at mutation-selection balance	
	Trait and character model	Gaussian approximation	House-of-cards approximation
Unconditional evolvability averaged across characters, $\bar{e}^* =$ average unconditional evolvability, $\bar{e}$	$\frac{V_y}{2}\sum_{j=1}^{n}\left(b_{1j}^2+b_{2j}^2\right)$	$\frac{\sigma_m}{2}\sum_{j=1}^{n}\frac{b_{1j}^2+b_{2j}^2}{\sqrt{s_1b_{1j}^2+s_2b_{2j}^2}}$	$2u\sum_{j=1}^{n}\frac{b_{1j}^2+b_{2j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}$
Conditional evolvability averaged across characters, $\bar{c}^*$	$\bar{e}^*\left(1-\frac{\left(\sum_{j=1}^{n}b_{1j}b_{2j}\right)^2}{\sum_{j=1}^{n}b_{1j}^2\sum_{j=1}^{n}b_{2j}^2}\right)$	$\bar{e}^*\left(1-\frac{\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{\sqrt{s_1b_{1j}^2+s_2b_{2j}^2}}\right)^2}{\sum_{j=1}^{n}\frac{b_{1j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}}\right)$	$\bar{e}^*\left(1-\frac{\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{s_1b_{1j}^2+s_2b_{2j}^2}\right)^2}{\sum_{j=1}^{n}\frac{b_{1j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}}\right)$
Average conditional evolvability, $\bar{c}$ $\left(=\sqrt{Det[\mathbf{BVB}^T]}\right)$	$V_y\sqrt{\sum_{j=1}^{n}b_{1j}^2\sum_{j=1}^{n}b_{2j}^2-\left(\sum_{j=1}^{n}b_{1j}b_{2j}\right)^2}$	$\sigma_m\sqrt{\sum_{j=1}^{n}\frac{b_{1j}}{\sqrt{s_1b_{1j}^2+s_2b_{2j}^2}}^2\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}-\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{\sqrt{s_1b_{1j}^2+s_2b_{2j}^2}}\right)^2}$	$4u\sqrt{\sum_{j=1}^{n}\frac{b_{1j}}{s_1b_{1j}^2+s_2b_{2j}^2}^2\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1b_{1j}^2+s_2b_{2j}^2}-\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{s_1b_{1j}^2+s_2b_{2j}^2}\right)^2}$

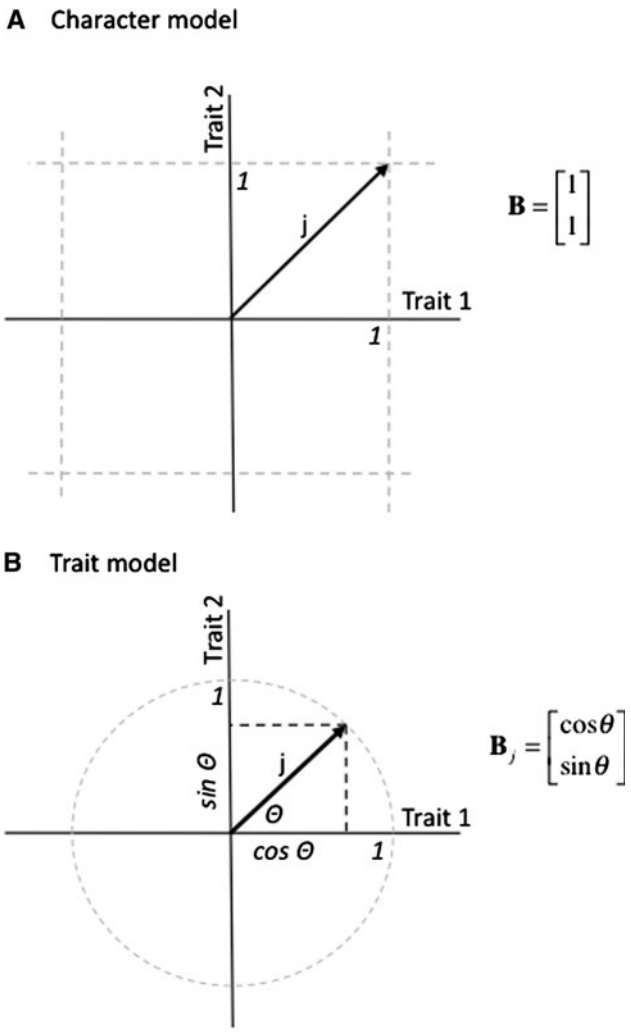

Fig. 1 Schematic representation of the two models of pleiotropy for two phenotypic characters. A locus is represented by a column vector in the **B** matrix and a vector in two-dimensional morphospace. **a** In the character model, the total effect of the locus is the length of the vector, while the effect of the pleiotropic locus per phenotypic character is independent of the degree of pleiotropy. Whether character 1, character 2, or both are affected, the effect per character is constant. **b** In the trait model the total effect of the locus is constant, as reflected in the constant vector length. The effect per trait depends on the degree of pleiotropy (i.e., the angle θ of the effect vector)

selection. Results on unconditional evolvabilities are presented briefly at the end.

Equal Molecular Variances at the Loci

All evolvabilities increase as the effects at the loci increase and there is no formal maximum in the absence of limits on the gene effects. Rather the maximal evolvability is determined by the maximum variance set in the model. Fixing the molecular variances in Eqs. (8) and (9) to unity we get the following expressions for the average conditional evolvabilities:

$$\bar{c}^* = \frac{1}{2}\sum_{j=1}^{n}\left(b_{1j}^2 + b_{2j}^2\right)\left(1 - \frac{\left(\sum_{j=1}^{n} b_{1j}b_{2j}\right)^2}{\sum_{j=1}^{n} b_{1j}^2 \sum_{j=1}^{n} b_{2j}^2}\right),$$

$$\bar{c} = \sqrt{\sum_{j=1}^{n} b_{1j}^2 \sum_{j=1}^{n} b_{2j}^2 - \left(\sum_{j=1}^{n} b_{1j}b_{2j}\right)^2}.$$

As is apparent above (see also Table 1), both types of conditional evolvability are maximized by increasing the variance of both characters and decreasing their covariance. In the character model this is achieved by full pleiotropy with hidden effects, in which all loci contribute to both characters, with no net covariance. The proof is given in Appendix 1. It follows that the most evolvable **B** matrix lacks modular effects under the character model. A such genotype-phenotype map is depicted in Fig. 2 for a system with two loci. In the trait model the sum of the effects on the two traits is constant, so maximization of average conditional evolvability requires only minimizing the contribution to covariance. This is minimized when the effects of pairs or groups of loci cancel out, and includes modular as well as hidden-pleiotropic genotype-phenotype maps. Multiple **B** matrices, with widely varying patterns of pleiotropy, exist that fulfill these general conditions (Fig. 2b). The corresponding **G** matrix has equal eigenvalues. The proof is given in Appendix 1.

We conclude that given equal variances across underlying loci, the reduction of pleiotropy is not an essential feature of genotype-phenotype maps that maximize evolvability, as measured by the amount of independent variance available to selection per character. In the case of the character model, modular genotype-phenotype maps even result in lower evolvability than the fully pleiotropic maps. All the corresponding **G** matrices that maximize evolvability are diagonal with equal variances per trait.

Mutation-Selection Balance: House-of-Cards Approximation of Equilibrium Variance

Average conditional evolvabilities are derived by substituting the equilibrium locus variance under the House-of-cards approximation (Eq. 1 into Eqs. 8, 9):

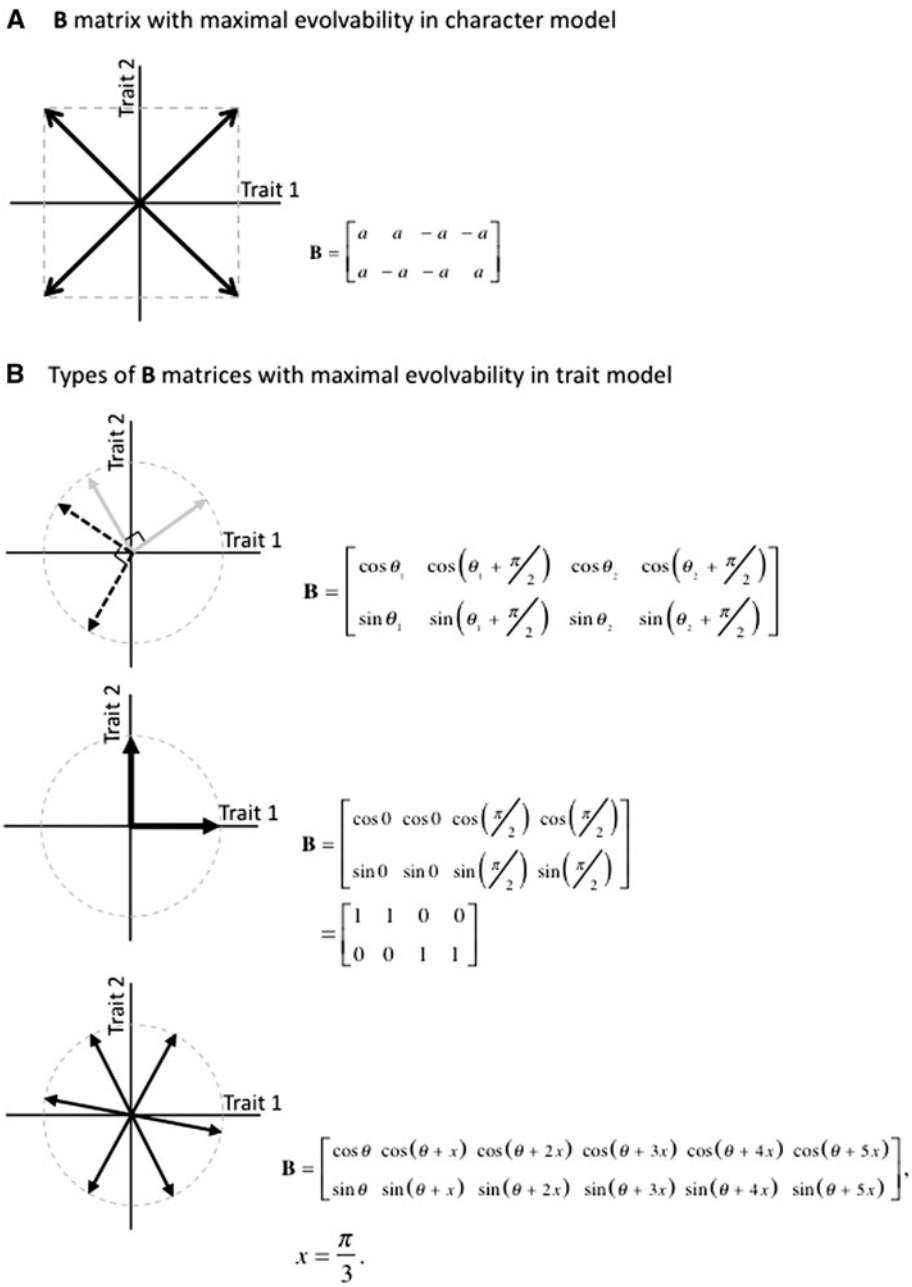

Fig. 2 The examples of vector arrangements characteristic of the GP maps with maximal average conditional evolvability, given equal variances at loci. Each vector represents an effect of the locus on the two-dimensional phenotype. The arrangement of vectors determines the distribution of variance among characters. The corresponding **B** matrices are shown in which each vector appears as a column. **a** In the character model, the most evolvable GP maps are characterized by vectors that affect both traits, generating variance along both trait axes, while covariance cancels out due to opposite effects. **b** In the trait model, the family of most evolvable GP maps is characterized by zero covariance, which can be reached by different arrangements of vectors. The first example shows pairs of orthogonal vectors, arbitrarily arranged relative to each other. The middle example shows a modular arrangement, which is a special case of the first arrangement, when pairs of orthogonal vectors come to be arranged on top of one another. The last example shows equally spaced vectors (Appendix 2)

$$\bar{c}^* = 2u\left[\sum_{j=1}^{n}\left(\frac{b_{1j}^2 + b_{2j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}\right)\left(1 - \frac{\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{s_1 b_{1j}^2 + s_2 b_{2j}^2}\right)^2}{\sum_{j=1}^{n}\frac{b_{1j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}}\right)\right],$$

$$\bar{c} = 4u\sqrt{\sum_{j=1}^{n}\frac{b_{1j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}\sum_{j=1}^{n}\frac{b_{2j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2} - \left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{s_1 b_{1j}^2 + s_2 b_{2j}^2}\right)^2}.$$

As the reduction of covariance between traits is crucial to increase conditional evolvability, we consider in the following two extreme **B** matrices that lead to zero covariance: (1) fully hidden-pleiotropic **B** matrix, in which all loci are pleiotropic and the synergistic and antagonistic effects cancel each other out, and (2) fully-balanced modular matrices, in which all loci affect only a single trait and the effects per trait are equal among traits. In these cases the trait-based average conditional evolvabilities become:

$$\bar{c}^*_{\text{mod}} = \frac{nu(s_1+s_2)}{s_1 s_2},$$

$$\bar{c}^*_h = \frac{4nu}{s_1+s_2},$$

$$\bar{c}^*_{\text{mod}} > \bar{c}^*_h, \quad \text{for all} \quad s_1 \neq s_2,$$

where "mod" stands for balanced modular case, and "h" stands for fully hidden pleiotropic, and n is the number of loci.

The conditional evolvabilities averaged over all directions are:

$$\bar{c}_{\text{mod}} = \frac{2nu}{\sqrt{s_1 s_2}},$$

$$\bar{c}_h = \frac{4nu}{s_1+s_2}.$$

$$\bar{c}_{\text{mod}} > \bar{c}_h, \quad \text{for all} \quad s_1 \neq s_2.$$

Note that for a given total selection strength $s_1 + s_2$, the product $s_1 s_2$ in nominator is highest when $s_1 = s_2$ and decreases with increasingly asymmetric selection, leading to increased conditional evolvability.

When the stabilizing selection on both characters is equally strong ($s_1 = s_2$), the modular and hidden pleiotropic genotype-phenotype maps result in an equal **G** matrix with the conditional evolvabilities: $\bar{c}^*_{\max} = \bar{c}_{\max} = 2nu/s$. In all other cases, both trait-based and averaged conditional evolvabilities are higher in modular maps.

Interestingly, the trait and character models of pleiotropy contribute equally to the two characters in this case. The proof is presented in Appendix 3, section 1. The two models can therefore be treated jointly. Furthermore, the contribution of a pleiotropic locus to the variance of a particular character is always smaller than the contribution of a locus that is modular for that trait. To see this, consider a modular locus with the **B** matrix column $\mathbf{b}_j = \begin{pmatrix} \pm 1 \\ 0 \end{pmatrix}$. Its contribution to the character variance is $4u/s_1$, while a pleiotropic locus with $\mathbf{b}_j = \begin{pmatrix} \pm\cos\theta \\ \pm\sin\theta \end{pmatrix}$ contributes the variance $G_{11j} = \frac{4u\cos^2\theta}{s_1\cos^2\theta + s_2\sin^2\theta}$.

In summary, under house-of-cards conditions (see discussion), modular and fully hidden pleiotropic genotype-phenotype maps are equally evolvable only when stabilizing selection is equally strong in all directions. In this case, all **B** matrices that generate zero net genetic covariance and equal genetic variances of both characters (whether modular, hidden pleiotropic or any balanced combination of the two) generate maximal average conditional evolvability for the given amount of variance and strength of selection. The higher number of loci that affect each trait compensates for the fact that the pleiotropic loci contribute less variance per trait. Under any unequal strength of stabilizing selection on the two characters, the modular genotype-phenotype map manifests higher average conditional evolvabilities.

Mutation-Selection Balance: Gaussian Approximation of Equilibrium Variance

The average conditional evolvabilities are derived by substituting the equilibrium locus variance under the Gaussian approximation (Eq. 2 into Eqs. 8, 9):

$$\bar{c}* = \frac{\sigma_m}{2}\left[\sum_{j=1}^{n}\frac{b_{1j}^2+b_{2j}^2}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}\left(1-\frac{\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}\right)^2}{\sum_{j=1}^{n}\frac{b_{1j}^2}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}\sum_{j=1}^{n}\frac{b_{2j}^2}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}}\right)\right],$$

$$\bar{c} = \sigma_m\sqrt{\sum_{j=1}^{n}\frac{b_{1j}^2}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}\sum_{j=1}^{n}\frac{b_{2j}^2}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}-\left(\sum_{j=1}^{n}\frac{b_{1j}b_{2j}}{\sqrt{s_1 b_{1j}^2+s_2 b_{2j}^2}}\right)^2}.$$

Table 2 Comparison between evolvabilities in fully-balanced modular and hidden-pleiotropic GP maps for different assumptions about the variance distribution at underlying loci

Optimized criterion	Uniform variances at loci		Variances at mutation-selection balance	
	Trait model	Character model	Gaussian approximation	House-of-cards approximation
Average evolvability of characters, $\bar{e}^*$ = average unconditional evolvability, $\bar{e}$ = conditional evolvability averaged across characters, $\bar{c}^*$				
Balanced modularity	$\frac{V_y n}{2}$	$\frac{V_y n}{2}$	$\frac{n\sqrt{u\gamma}\left(\sqrt{s_1}+\sqrt{s_2}\right)}{4\sqrt{s_1 s_2}}$	$\frac{nu(s_1+s_2)}{s_1 s_2}$
Hidden pleiotropy	$\frac{V_y n}{2}$	$V_y n$	$\frac{n\sqrt{u\gamma}}{\sqrt{s_1+s_2}}$	$\frac{4nu}{s_1+s_2}$
Average conditional evolvability, $\bar{c}$				
Balanced modularity	$\frac{V_y n}{2}$	$\frac{V_y n}{2}$	$\frac{n\sqrt{u\gamma}}{2\sqrt[4]{s_1 s_2}}$	$\frac{2nu}{\sqrt{s_1 s_2}}$
Hidden pleiotropy	$\frac{V_y n}{2}$	$V_y n$	$\frac{n\sqrt{u\gamma}}{\sqrt{s_1+s_2}}$	$\frac{4nu}{s_1+s_2}$

Evolvabilities are presented for n loci

Table 3 The summary of the conditions under which the modular or the hidden pleiotropic GP maps yield higher evolvability

	Equal variances		Variances at mutation-selection balance	
	Trait model	Character model	Gaussian approximation	House-of-cards approximation
$\bar{e}^*$	$\bar{e}^*_{\text{mod}} = \bar{e}*_h$	$\bar{e}^*_{\text{mod}} < \bar{e}^*_h$	$\bar{e}^*_{\text{mod}} > \bar{e}^*_h \Leftrightarrow (s_1-s_2)^2 > 4s_1s_2$ $\bar{e}^*_{\text{mod}} < \bar{e}^*_h \Leftrightarrow (s_1-s_2)^2 < 4s_1s_2$	$\bar{e}^*_{\text{mod}} > \bar{e}^*_h \Leftrightarrow s_1 \neq s_2$ $\bar{e}^*_{\text{mod}} = \bar{e}^*_h \Leftrightarrow s_1 = s_2$
$\bar{e}$	$\bar{e}_{\text{mod}} = \bar{e}_h$	$\bar{e}_{\text{mod}} < \bar{e}_h$	$\bar{e}_{\text{mod}} > \bar{e}_h \Leftrightarrow (s_1-s_2)^2 > 4s_1s_2$ $\bar{e}_{\text{mod}} < \bar{e}_h \Leftrightarrow (s_1-s_2)^2 < 4s_1s_2$	$\bar{e}_{\text{mod}} > \bar{e}_h \Leftrightarrow s_1 \neq s_2$ $\bar{e}_{\text{mod}} = \bar{e}_h \Leftrightarrow s_1 = s_2$
$\bar{c}^*$	$\bar{c}^*_{\text{mod}} = \bar{c}^*_h$	$\bar{c}^*_{\text{mod}} < \bar{c}^*_h$	$\bar{c}^*_{\text{mod}} > \bar{c}*_h \Leftrightarrow (s_1-s_2)^2 > 4s_1s_2$ $\bar{c}^*_{\text{mod}} < \bar{c}^*_h \Leftrightarrow (s_1-s_2)^2 < 4s_1s_2$	$\bar{c}^*_{\text{mod}} > \bar{c}^*_h \Leftrightarrow s_1 \neq s_2$ $\bar{c}^*_{\text{mod}} = \bar{c}^*_h \Leftrightarrow s_1 = s_2$
$\bar{c}$	$\bar{c}_{\text{mod}} = \bar{c}_h$	$\bar{c}_{\text{mod}} < \bar{c}_h$	$\bar{c}_{\text{mod}} > \bar{c}_h \Leftrightarrow (s_1-s_2)^2 > 12s_1s_2$ $\bar{c}_{\text{mod}} < \bar{c}_h \Leftrightarrow (s_1-s_2)^2 < 12s_1s_2$	$\bar{c}_{\text{mod}} > \bar{c}_h \Leftrightarrow s_1 \neq s_2$ $\bar{c}_{\text{mod}} = \bar{c}_h \Leftrightarrow s_1 = s_2$

We again focus on the fully modular, and the fully hidden pleiotropic genotype-phenotype map. For these maps the conditional evolvabilities averaged over traits are:

$$\bar{c}^*_{\text{mod}} = \frac{n\sigma_m\left(\sqrt{s_1}+\sqrt{s_2}\right)}{4\sqrt{s_1 s_2}},$$

$$\bar{c}^*_h = \frac{n\sigma_m}{\sqrt{s_1+s_2}},$$

$$\bar{c}^*_{\text{mod}} < \bar{c}^*_h \Leftrightarrow (s_1-s_2)^2 < 4s_1s_2,$$

$$\bar{c}^*_{\text{mod}} > \bar{c}^*_h \Leftrightarrow (s_1-s_2)^2 > 4s_1s_2.$$

The hidden-pleiotropic genotype-phenotype map is thus more evolvable, except when the strengths of stabilizing selection on the two traits differ strongly. At equal strengths of stabilizing selection shaping the underlying variance of both traits, the average conditional evolvability of the two maps is: $\bar{c}^*_{\text{mod}} = \frac{n\sigma_m}{2\sqrt{s}}$, and $\bar{c}^*_h = \frac{n\sigma_m}{\sqrt{2s}}$.

The conditional evolvabilities of modular and hidden-pleiotropic maps averaged across all directions in morphospace are

$$\bar{c}_{\text{mod}} = \frac{n\sigma_m}{2\sqrt[4]{s_1 s_2}},$$

$$\bar{c}_h = \frac{n\sigma_m}{\sqrt{s_1+s_2}},$$

$$\bar{c}_{\text{mod}} < \bar{c}_h \Leftrightarrow (s_1-s_2)^2 < 12s_1s_2,$$

$$\bar{c}_{\text{mod}} > \bar{c}_h \Leftrightarrow (s_1-s_2)^2 > 12s_1s_2.$$

Again, the genotype-phenotype map with hidden pleiotropy is the more evolvable, except if stabilizing selection is very weak on one of the traits. At equal strengths of stabilizing selection on the traits at equilibrium, $\bar{c}_{\text{mod}} = \frac{n\sigma_m}{2\sqrt{s}}$, and $\bar{c}_h = \frac{n\sigma_m}{\sqrt{2s}}$.

Figure 3a shows the parts of the parameter space (s_1, s_2) for which either the modular or the pleiotropic genotype-phenotype map has the higher average conditional evolvability.

As under house-of-cards approximation, it can be shown that the two pleiotropic models (trait and character) are indistinguishable with respect to the genetic variation contributed per trait (proof in Appendix 3, section 2). Furthermore, also under the Gaussian approximation the

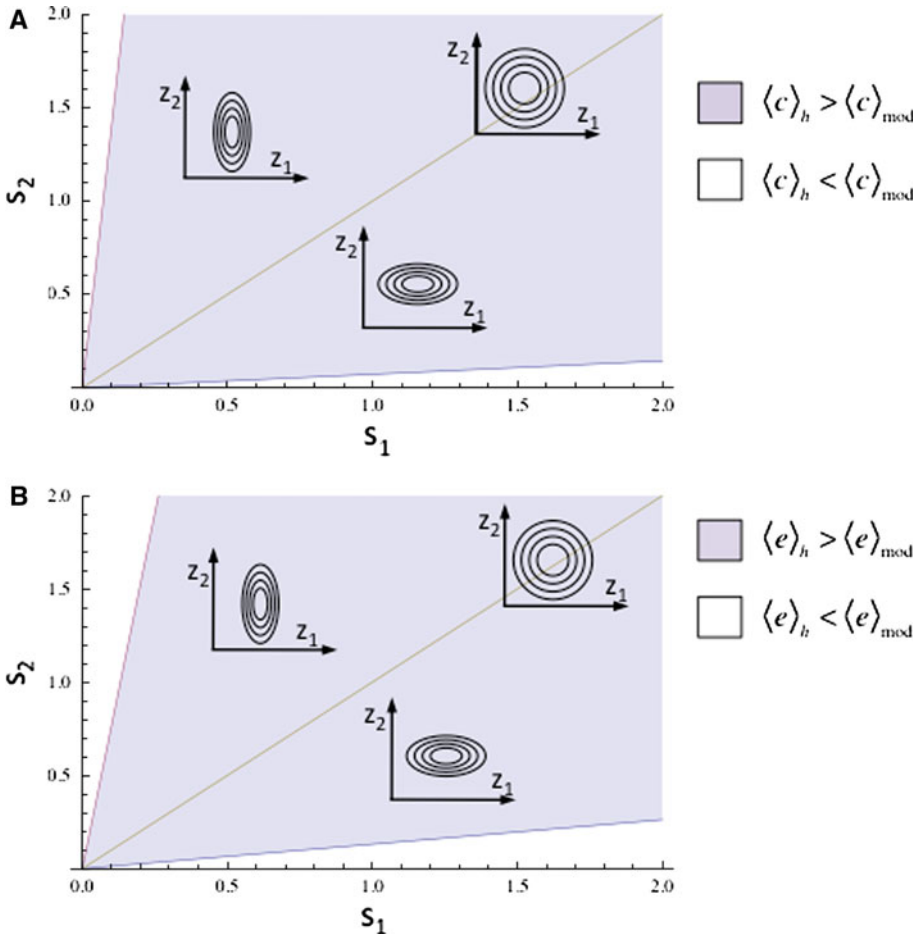

Fig. 3 Both plots show the parameter space defined by the strength of stabilizing selection on both characters that determine the variance distribution at the mutation-selection equilibrium. The *small plots* show the bivariate phenotypic distributions corresponding to the combination of strengths of selection in particular parts of the graph. The *shaded regions* show the combinations of parameter values (and hence the distribution of variance), at which genotype-phenotype maps with hidden pleiotropy are more highly evolvable than those with modular pleiotropy when the criterion is conditional evolvability (**a**), or unconditional evolvability (**b**)

pleiotropic locus always contributes less variance to each character than a modular locus: the trait variance generated by a pleiotropic locus $\left(\frac{\sigma_m \cos^2\theta}{\sqrt{s_1\cos^2\theta + s_2 \sin^2\theta}}\right)$ is always smaller than that of the modular locus $\left(\frac{\sigma_m}{\sqrt{s_1}}\right)$.

It follows that under the conditions appropriate for the Gaussian approximation, the fully hidden-pleiotropic structure maximizes average conditional evolvability, except when stabilizing selection on the traits at mutation-selection equilibrium is highly asymmetrical. Even though the contribution of a single locus to variance is lesser when it affects another character, this does not outweigh the fact that every locus contributes to the variance of the character at full pleiotropy, while only part of the loci contribute when genotype-phenotype map is modular.

Maximizing Unconditional Evolvability

Unconditional evolvability differs from conditional evolvability in that the amount of covariance between traits is irrelevant (see summary in Table 3). Maximizing average unconditional evolvability only depends on maximizing variance. Unconditional evolvability averaged across all directions and across the two orthogonal characters yields the same results ($\bar{e} = \bar{e}^*$).

When the molecular variance is uniform across loci, the two pleiotropic models differ in their optimal arrangement of genetic effects. Under the trait model, all genotype-phenotype maps have equal total amount of variance, and hence equal evolvability. Under the character model, the average unconditional evolvability is maximized when all loci contribute maximally to the variance of all traits, and hence when all loci have maximally pleiotropic effects. If variance is kept in mutation-selection equilibrium under the house-of-cards conditions, the average unconditional evolvability is always equal to or higher in modular maps than in genotype-phenotype maps with hidden pleiotropy:

$$\bar{e} = \bar{e}^* = 2u \sum_{J=1}^{n} \frac{b_{1j}^2 + b_{2j}^2}{s_1 b_{1j}^2 + s_2 b_{2j}^2}.$$

$$\bar{e}_{\text{mod}} = nu \frac{s_1 + s_2}{s_1 s_2},$$

$$\bar{e}_h = \frac{4nu}{s_1 + s_2}.$$

$$\bar{e}_{\text{mod}} > \bar{e}_h, \quad \text{for all} \quad s_1 \neq s_2.$$

Only in the special case when stabilizing selection is equally strong on both traits ($s_1 = s_2$) will the modular and full hidden pleiotropic matrices have equal evolvabilities. In this case, the average evolvability is $\bar{e} = 2nu/s$. As the signs of pleiotropic effects are irrelevant, the above pleiotropic matrices are representative for all pleiotropic matrices.

Under the Gaussian conditions, however, the average unconditional evolvability is maximized by hidden pleiotropic genotype-phenotype maps under most combinations of stabilizing selection on the two characters (Fig. 3b).

$$\bar{e} = \bar{e}^* = \frac{\sigma_m}{2}\sum_{j=1}^{n}\frac{b_{1j}^2 + b_{2j}^2}{\sqrt{s_1 b_{1j}^2 + s_2 b_{2j}^2}}.$$

$$\bar{e}_{\mathrm{mod}} = n\sigma_m \frac{\sqrt{s_1} + \sqrt{s_2}}{4\sqrt{s_1 s_2}},$$

$$\bar{e}_h = \frac{n\sigma_m}{\sqrt{s_1 + s_2}}.$$

$$\bar{e}_{\mathrm{mod}} < \bar{e}_h \Leftrightarrow (s_1 - s_2)^2 < 4s_1 s_2,$$

$$\bar{e}_{\mathrm{mod}} > \bar{e}_h \Leftrightarrow (s_1 - s_2)^2 > 4s_1 s_2.$$

If the stabilizing selection on the two traits is symmetrical, the hidden pleiotropic genotype-phenotype map is always more evolvable than the modular genotype-phenotype map ($\bar{e}_{\mathrm{mod}} = \frac{n\sigma_m}{2\sqrt{s}}$, $\bar{e}_h = \frac{n\sigma_m}{\sqrt{2s}}$; $\bar{e}_h > \bar{e}_{\mathrm{mod}}$). As the presence of covariance is irrelevant when maximizing average unconditional evolvability, the above therefore holds for fully pleiotropic genotype-phenotype maps in general.

Discussion

The purpose of this work was to find genotype-phenotype maps that maximize the potential to respond to a selective challenge under different assumptions about the underlying molecular variance. In particular, we have shown that a low degree of pleiotropy (i.e., modularity) is not a universal characteristic of the genotype-phenotype maps that maximize evolvability. In fact, if there is no constraining selection and all genes have equal variation, the highest evolvability is achieved when all genes affect all characters, as this maximizes the amount of genetic variation along any specific direction in morphospace. If there is constraining selection, however, the picture is more complex. The conditional evolvability measures ability to respond in a specific direction when there is stabilizing selection along other directions. This measure is optimized by reducing genetic covariances as much as possible and by increasing and evening out genetic variances as much as possible. These demands may be in conflict, and the degree to which this conflict can be resolved depends on what constraints exist on the possible patterns of pleiotropy. Hansen (2003) found that when only one type of positive pleiotropy was possible, then the most evolvable maps were the ones in which 16% of genetic variation was pleiotropic. In the present paper we have looked at situations where all kinds of pleiotropy are possible. Then it is clear that the most evolvable maps are to be found among those that can generate a diagonal **G**-matrix in which all genetic covariances are zero. Modular genotype-phenotype maps can achieve this, but so can patterns of variable pleiotropy that cancels out on average (hidden pleiotropy). The detailed structure of the most evolvable genotype-phenotype map depends on the initial distribution of variance at the underlying loci. When the variance at the underlying loci is assumed to be uniform across loci, maps with fully hidden pleiotropic effects, which generate no genetic covariance while contributing fully to the variance of all characters, yield equal or higher average conditional evolvability than any modular genotype-phenotype map. At mutation-selection equilibrium, the distribution of variance is determined by the mutation rate and the strengths of stabilizing selection on both characters. When the equilibrium variance distribution is approximated by the house-of cards approximation, *any* asymmetry in the strength of stabilizing selection on the two characters results in modular genotype-phenotype maps yielding higher evolvability. When equilibrium variance can be approximated by the Gaussian approximation, however, then hidden pleiotropic genotype-phenotype maps yield higher evolvability under most conditions; the exception being when the strength of stabilizing selection on the two characters is extremely unequal. The Gaussian and house-of-cards approximations are valid under different conditions (Burger 2000). The Gaussian approximation is based on high mutation rates and small phenotypic effects of mutations (Turelli 1984; Burger and Hofbauer 1994), whereas the house-of-cards approximation is valid when the mutation rates are lower and the phenotypic variance of the new mutations is greater than the equilibrium variance (Burger and Hofbauer 1994). Based on this, our results imply that under high mutation rates, pleiotropic genotype-phenotype maps yield higher evolvability across most combinations of stabilizing selection, whereas with lower mutation rates, modular genotype-phenotype maps yield higher levels of evolvability (see Table 3 for summary). This is an intuitively plausible result, as in the populations where single mutations contribute relatively little variation, the disadvantages of pleiotropic mutations are less important and may be compensated additively by existing variation; whereas in a situation approximated by house-of-cards model, large pleiotropic mutations overwhelming existing variation may be more disadvantageous (Fig. 4).

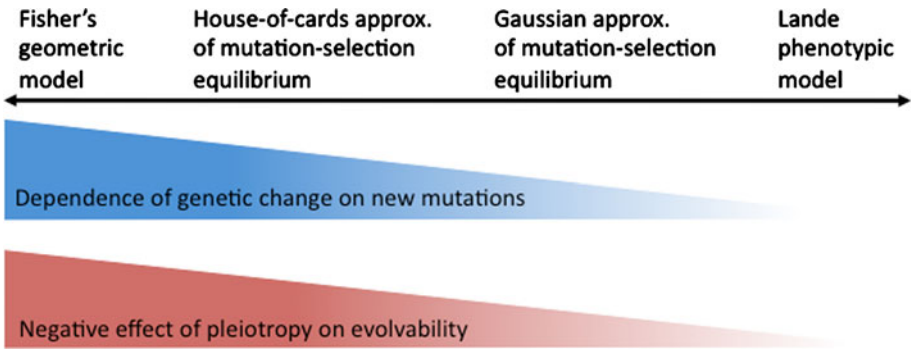

Fig. 4 The models of evolutionary change on a conceptual continuum, with regard to the assumptions about the relative importance of mutational and standing variation. Correspondingly, the negative effect of pleiotropy is greater in models emphasizing mutational variance than in those emphasizing the standing variation as the raw material for selection

We investigated two sets of constraints on what patterns of pleiotropy are possible. In the character model, we assumed that the effect a gene can have on one trait is unrelated to the effects it has on the other trait. This leads to a situation in which the largest vector effects can occur when both traits are equally affected (with equal or opposite sign). This is a situation that could favor pleiotropy, as more variation is possible in directions of morphospace where both characters change. Indeed, when the underlying molecular variances are uniform, we found the highest evolvability for maps with equal and hidden pleiotropy. In the trait model, we assumed that the length of the effect vector had a constant maximum, so that no direction in morphospace was favored. In this case, a range of fully modular and fully hidden maps had equal effects on evolvability when underlying molecular variances were equal. Under the mutation-selection models, however, the difference between the character and the trait models disappeared, as less molecular variance was maintained when the genes had larger effects. This result resembles the Haldane-Muller principle, which states that the mutation load is independent of the mutational effect size (Haldane 1937; Muller 1950; Burger 2000).

We may think of the character axes in our models as representing functional combinations of traits (modules), and allele substitutions with effects along these axes can be interpreted as having functionally integrated effects on these traits. Modularity in our model would then represent Riedl's (1978) idea that genotype-phenotype maps are adaptively structured to generate variation along these functionally integrated modules (Wagner and Laubichler 2004). Without going into the question of how such an adaptation may arise, our results are relevant to this idea, because they show that a pleiotropic modular organization is not the only way to eliminate selective constraints across modules and to maximize evolvability. Classical modularity is indeed a highly evolvable solution in many situations, but it is not always the best solution. In particular, under conditions of high mutation rates or equal variances across loci, genotype-phenotype maps with hidden pleiotropy are more evolvable.

If evolvability is the ability to respond to a selective challenge, then we may expect that the evolvability will depend on the type of selection. We considered two types of selective challenge. When maximizing the conditional evolvability along the two character axes, we effectively assumed that selection would only seek to change one character at the time with the other character under stabilizing selection. Under this regime the fully hidden pleiotropic genotype-phenotype map is only equivalent to the modular map when the molecular variances of all loci are equal, which under mutation-selection balance occurs when stabilizing selection and mutation rates are equally strong on the two traits. With any asymmetry, a modular map can generate higher evolvability. When we maximized conditional evolvability as an average over any combination of character values, we effectively assumed that directional selection is equally likely in any direction of morphospace. In this case, complementary pleiotropic maps can generate higher evolvability than the purely modular maps under conditions where mutation rates are high and stabilizing selection not strongly asymmetrical, while modular maps are still best at lower mutation rates and asymmetric stabilizing selection.

Our measures of evolvability are highly schematic in that they are derived from single-generation rates of evolution when adaptive and constraining selection are at balance (Hansen et al. 2003a). Pleiotropy has consequences that are not apparent in the multivariate single-generation response to selection. One such consequence is on the stability of genetic correlation over time. The ultimate genetic covariance matrix, determining the response to selection, is determined by the structure of genotype-phenotype map *and* by the allele frequencies. With a modular genotype-phenotype map, changes in allele frequencies cannot generate covariance except through linkage

disequilibrium, while non-zero covariances could easily evolve from an initially hidden-pleiotropic structure. Allele-frequency changes in presence of hidden pleiotropy may therefore cause instability of the **G**-matrix in the long term. On this basis, we hypothesize that modularity of the pleiotropic effects has higher long-term advantage if generating genetic correlations by allele-frequency change is highly deleterious, as for example in traits that are always exposed to separate selection regimes. On the contrary, pleiotropic genotype-phenotype maps may be advantageous if there are frequent changes in the trait combinations preferred by selection. In short, a higher degree of pleiotropy increases the possibilities to move in the phenotypic space, but also introduces instability of genetic correlations over time. The details of how allele frequencies and selection regimes affect the long-term optimality of different genotype-phenotype maps require further study.

Baatz and Wagner (1997) demonstrated a further effect of pleiotropy beyond its effect on genetic correlation. They showed that pleiotropy might hamper the response to selection even when it generates no net genetic correlation. This is because selection on the mean of one character may affect the variance of the second character, even when having no effect on its mean. If the selection on one character increases the variance of the second character that is under stabilizing selection, this effect still imposes a constraint to selection in spite of absence of genetic correlation between their means. In the opposite case, when selection on the character reduces the variance of the character under stabilizing selection, the response to selection is enhanced.

Another potential effect of pleiotropy that escapes the model used here involves epistasis. The **B** matrix represents a linear genotype-phenotype map. Nevertheless, epistasis is a common finding in empirical studies across species (e.g., Wolf et al. 2000; Cheverud et al. 2001; Bradshaw et al. 2005; Brem and Kruglyak 2005; Malmberg et al. 2005; West et al. 2007; Le Rouzic et al. 2008; Pavlicev et al. 2010). So far modeling the effects of epistasis on evolvability has been restricted to the univariate case (Hermisson et al. 2003; Carter et al. 2005; Hansen et al. 2006; but see Hansen and Wagner 2001). When epistasis affects pleiotropic genes, it can change variances and covariances of traits in a variety of ways, depending on exactly how gene substitutions modify the effects of each other (Hansen and Wagner 2001; Cheverud et al. 2004; Carter et al. 2005; Hansen 2006; Wolf et al. 2006; Pavlicev et al. 2008, 2010, 2011a, b). Expanding the existing study of effects of epistasis on evolvability to the multivariate case will reveal to what extent epistasis may allow pleiotropy to evolve in a manner that could increase evolvability, or alternatively, decrease evolvability through canalization.

Our results are derived for a two-dimensional phenotype. With higher-dimensional phenotypes one may speculate that constrains could arise from complete lack of variation in some directions. Also, with large number of traits, the instability due to allele-frequency change may increase, as it is harder to simultaneously balance covariances of multiple traits at full pleiotropy. Extrapolating the approach used in this work and assuming an uniform distribution of molecular variance, we may expect that reduction of variance per character in the modular genotype-phenotype maps (and character model), as compared to the hidden pleiotropic maps, is stronger when the total variance is divided among more than two phenotypic characters. This could lead to an advantage of pleiotropy over modularity. When the underlying distribution of variance across loci is determined by mutation-selection equilibrium the situation is more complex, a question that will require more attention in the future.

By focusing on conditional evolvabilities averaged across directions of morphospace, we implicitly assumed that selection is equally likely in those directions. If selection is more likely along certain axes in morphospace, the situation can be formulated in terms of this study by using the axes of selective importance as character axes, and applying the conditional evolvability averaged across these axes. Pleiotropy can then be defined with respect to these axes.

Among experimental studies, support can be found for very broad as well as very restricted and modular pleiotropy. It is becoming clear that quantitative characters are often affected by a very large number of genes, many of which may remain undetected by the conventional studies, as is also suggested by a high proportion of unexplained genetic variance (Eichler et al. 2010; Yang et al. 2010). Given the limited number of genes in the genome, a high degree of polygeny means that most genes must affect many characters, and hence that the genes underlying any one character typically also have effects on many other characters (e.g., Walsh and Blows 2009; Edwards and Weinig 2011). This is further supported by the prevalence of high genetic correlation that we find between most characters (e.g., Kirkpatrick 2009). On the other hand, low average degrees of pleiotropy (Gu 2007; Albert et al. 2008; Wagner et al. 2008; Zou et al. 2008; Su et al. 2010), or actual modularity of gene action (Mezey et al. 2000; Brem et al. 2002; Ehrich et al. 2003; Cheverud et al. 2004; Albertson et al. 2005; Juenger et al. 2005; Hlusko et al. 2011) are also common findings of empirical studies. We note that a low average degree of pleiotropy is not necessarily equivalent to modularity, as the phenotypic domains might not coincide among genes, generating any degree of covariance. Clearly, some empirical results may be artifacts of the methods. For example, the pleiotropy of loci

segregating in an intercross of the selected lines used for gene mapping may have been influenced by selection. Also, pleiotropy may differ between the detectable loci and those with small effects (Klingenberg et al. 2004). Furthermore, the choice of measurements to assess pleiotropy may further bias the results. In spite of the methodical biases however, it is still an open question how these contradicting results on the degree of pleiotropy can be reconciled.

The structure and prevalence of most evolvable genotype-phenotype maps are interesting regardless of whether this structure can be directly selected for. However, the evolution of the genotype-phenotype maps is a central issue and there is an ongoing debate on the effectiveness of direct selection for evolvability (e.g., Hansen 2003; Proulx and Phillips 2005; Hansen 2006; Lynch 2007a, b, c; Wagner et al. 2007; Draghi and Wagner 2008; Fierst 2011; Hansen 2011; Pavlicev et al. 2011a). Theoretical models indicate the potential for selection on evolvability, but some authors argue that direct selection for evolvability is not feasible, or at least not necessary to explain organismal or genomic structure (Lynch 2007a, b, c; Hansen 2011). Experimental work directly addressing the evolution of the genotype-phenotype map by selection can illuminate this question, but has so far been rare (but see, e.g., Allen et al. 2008; Delph et al. 2011). Insight into this question can also be gained by studying the structure and evolvability of empirical genotype-phenotype maps, and by comparison to the result of this study. Are the empirical genotype-phenotype maps maximally evolvable? Provided there are no constraints on what patterns of pleiotropy are possible, we have shown that all maximally evolvable genotype-phenotype maps generate diagonal **G** matrices. Empirical **G** matrices are usually far from diagonal, and this may be because the measurements chosen do not represent biological characters as discussed above, or because genotype-phenotype maps are not evolutionary optimized for average evolvability in the two senses we have investigated. A strong genetic correlation means that genetic variation gets concentrated along certain axes in morphospace, and we can not rule out that the genotype-phenotype map has been molded to generate high evolvability along directions where evolutionary changes are more likely (Jones et al. 2007), or simply to minimize genetic load by reducing variation in directions that are not likely to be adaptive.

Evolvability has frequently been addressed by estimating dimensionality of genetic covariance matrices, i.e., by the number of independent dimensions that possess significant amount of genetic variance (Mezey and Houle 2005; Hine and Blows 2006; McGuigan and Blows 2007; Kirkpatrick 2009). The current study is distinct in that it emphasizes the distribution of genetic variance across dimensions and the underlying GP map producing the distribution, rather than exploring its sole net presence or absence in single dimensions. Whereas high-dimensional **G** matrices can result from genotype-phenotype maps with varying degrees of pleiotropy, low dimensionality reveals lack of genetic variance in some of the phenotypic directions and therefore necessarily also lack of pleiotropy involving these characters. Put into the context of dimensionality, our results show that the most evolvable genotype-phenotype maps have full dimensionality of **G**. This is not surprising when average evolvabilities across all directions of morphospace are the criterion to be maximized. Optimizing a genotype-phenotype map for a particular direction of selection rather than the average across all possibilities, would result in a low-dimensional **G** matrix with most variation aligned in the direction of selection, and in a correspondingly different genotype-phenotype map.

The effect of pleiotropy on evolution has also been addressed using approaches based on Fisher's (1958) geometric model (Kimura 1983; Orr and Coyne 1992; Hartl and Taubes 1998; Waxman and Welch 2003, 2005; Martin and Lenormand 2006). These studies differ from the present study in that they predominantly focus on the evolvability of a single trait (rather than including the constraining trait) and furthermore, are based on different assumptions about variation. The geometric approach assumes that the rate of evolution depends on the new arising mutational variation. In contrast, quantitative genetic models such as the present model, assume that the rate of evolution primarily depends on selection on existing variation. Due to different assumptions, the conclusions of the models are not directly comparable. Technically, both situations can be seen as simplifications, representing opposing extremes on a continuum combining arising and extant variation. The fact that the two approaches frequently arrive at different conclusions (e.g., compare Wagner 1988; Orr 2000) is itself interesting, as it implies that pleiotropy may have different effects in the two situations.

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Appendix 1

Here we present the derivation of the GP maps maximizing average conditional evolvability. The two models of pleiotropy are dealt with separately. We also present the maximization of the character conditional evolvability in the character model.

Character Model

Discrete Genetic Effects

In this part, we are concerned with discrete effects of genes, i.e., $b_{ij} \in \{-1, 0, 1\}$. Let the underlying genes have either a positive, a negative, or no effect on the trait, and let each trait be affected by at least one gene. We term modular loci (column vectors of **B**) as those affecting only one trait (i.e., (1,0), (−1,0), (0,1) or (0, −1)), synergistic loci as those affecting both traits in the same way ((1,1) or (−1, −1)), and antagonistic loci as those affecting both traits in opposite ways ((1, −1) or (−1,1)).

The squared average conditional evolvability $\bar{c}^2$ for two traits equals the sum of the products of determinants of all minors of **B** (a consequence of Cauchy-Binet formula). We will denote the 2 × 2 sub-matrices (minors) of **B** as $\mathbf{B_i}$, where $i \in \{2, \ldots, n(n-1)/2\}$ and n is the number of genes.

$$\begin{aligned}\bar{c}^2 &= Det\left[\mathbf{B}\mathbf{B}^{\mathbf{T}}\right] = Det[\mathbf{B_1}]Det\left[\mathbf{B}_{\mathbf{1}}^{\mathbf{T}}\right] + \cdots \\ &\quad + Det\left[\mathbf{B}_{n(n-1)/2}\right]Det\left[\mathbf{B}_{n(n-1)/2}\right] \\ &= \sum_{i}^{n(n-1)/2} (Det[\mathbf{B}_i])^2.\end{aligned}$$

Following the solution of Hadamard's maximum determinant problem, the maximum determinant of an m x m matrix with all entries $a_{ij} \leq |1|$ equals $m^{m/2}$, which is two in the case of a 2 × 2 matrix. It can be furthermore shown (Brenner and Cummings 1972; Ehlich 1964) that if matrix elements are restricted to discrete values (−1, 0, 1), there exist four distinct matrices with the maximum determinant (=2). These are, written by row: {{−1, −1}, {1, −1}}, {{−1, 1}, {−1, −1}}, {{−1, 1}, {1, 1}}, {{1, 1}, {1, −1}}. As we are interested in maximum squared values of the determinant, we also consider the minimum determinant (−2). The sign of the determinant changes by exchanging the rows or columns of a matrix, which results in four more optimal matrices. Note that all of these matrices combine one column vector with synergistic and one column vector with antagonistic effects. Hence, the problem of maximum conditional evolvability of a matrix with more than two vectors reduces to the problem of combining the column vectors of **B** such that they result in most optimal combination of determinants of submatrices $\mathbf{B_i}$. We present the values of the determinants of all four possible sub-matrices in Table 4.

Table 4 Summary of determinants of different types of 2 × 2 submatrices

2 × 2 Submatrix	Determinant
One column or row is a scalar multiply of another (includes equal vectors)	0
Two oppositely modular vectors	1
Any non-modular vector combined with a modular vector	1
All combinations of antagonistic and synergistic vectors	2

The solution can be found by considering the general equation for combining the four groups of loci (antagonistic, synergistic, two modular) in different proportions and determining the proportions at which the average conditional evolvability is the highest.

Let n_{M1}, n_{M2}, n_A, n_S be the counts of modular loci along the trait 1, modular loci along the trait 2, antagonistic and synergistic loci, respectively. Let x_{M1}, x_{M2}, x_A, and x_S be their respective proportions in the total number of loci n:

$x_{M1} = \frac{n_{M1}}{n}, x_{M2} = \frac{n_{M2}}{n}, x_A = \frac{n_A}{n}, x_S = \frac{n_S}{n}$; so that $x_{M1} + x_{M2} + x_A + x_S = 1$. Then $\bar{c}^2$ can be written as the number of times a particular type of submatrix occurs, times the value of its squared determinant (see Table 4):

$$\begin{aligned}\bar{c}^2 &= \left(\frac{(nx_{M1})^2 - nx_{M1}}{2}\right)0^2 + \left(\frac{(nx_{M2})^2 - nx_{M2}}{2}\right)0^2 \\ &\quad + \left(\frac{(nx_A)^2 - nx_A}{2}\right)0^2 + \left(\frac{(nx_S)^2 - nx_S}{2}\right)0^2 \\ &\quad + \left(n^2 x_{M1}x_{M2}\right)1^2 + \left(n^2 x_{M1}x_A\right)1^2 + \left(n^2 x_{M1}x_S\right)1^2 \\ &\quad + \left(n^2 x_{M2}x_A\right)1^2 + \left(n^2 x_{M2}x_S\right)1^2 + \left(n^2 x_S x_A\right)2^2\end{aligned}$$

where n is a constant and can be neglected. Maximizing the equation above under the constraint $x_{M1} + x_{M2} + x_A + x_S = 1$ yields a single solution $x_A = x_S = 0.5$ and consequently $x_{M1} = x_{M2} = 0$. The maximum value of the function $\bar{c}^2$ equals n^2; therefore the maximum average conditional evolvability equals n. This shows that the single combination of loci giving the highest average conditional evolvability is the one in which half of the loci have antagonistic and the other half synergistic effects on the two traits, but none are modular. The average evolvability of such GP map is n, hence max $\bar{c} = \bar{e}$. Also note that the average unconditional evolvability is at its maximum here, because all vectors attain their maximal length.

Continuous Genetic Effects

Here we show that the above is also the solution to the **B** matrix of continuous effects. We show that the values of the determinants in the above matrices are the maximal values and no other combination of effects will give higher average conditional evolvability.

Consider the type of submatrices above that yield a non-zero determinant. These are all submatrices combining loci with non-equal effects. With respect to the sign of the effect, we again classify the types of loci as before:

modular, synergistic and antagonistic. For each submatrix with a non-zero determinant, involving two different types of loci, we show that the determinant (*Det*) will decrease, as the elements of the submatrix deviate from the limits of the interval [−1,1] by some amount ε_i, where $0 \leq \varepsilon_i \leq 1$.

Then for a submatrix combining a modular and an antagonistic vector:

$$\begin{vmatrix} 0 & -1+\varepsilon_2 \\ 1-\varepsilon_1 & 1-\varepsilon_3 \end{vmatrix} = 1-\varepsilon_1-\varepsilon_2+\varepsilon_1\varepsilon_2 \Rightarrow 0 \leq Det \leq 1;$$

for a submatrix combining a modular and a synergistic vector:

$$\begin{vmatrix} 0 & 1-\varepsilon_2 \\ 1-\varepsilon_1 & 1-\varepsilon_3 \end{vmatrix} = \varepsilon_1+\varepsilon_2-\varepsilon_1\varepsilon_2-1 \Rightarrow -1 \leq Det \leq 0;$$

for a submatrix combining two modular vectors for different traits:

$$\begin{vmatrix} 1-\varepsilon_1 & 0 \\ 0 & 1-\varepsilon_3 \end{vmatrix} = \varepsilon_1+\varepsilon_2-\varepsilon_1\varepsilon_2-1 \Rightarrow -1 \leq Det \leq 0$$

and for a submatrix combining an antagonistic and a synergistic vector:

$$\begin{vmatrix} 1-\varepsilon_1 & -1+\varepsilon_3 \\ -1+\varepsilon_2 & 1-\varepsilon_4 \end{vmatrix} = 2-\varepsilon_1-\varepsilon_2-\varepsilon_3-\varepsilon_4+\varepsilon_2\varepsilon_3+\varepsilon_1\varepsilon_4 \Rightarrow 0 \leq Det \leq 2.$$

Thus, submatrix determinants are maximized when $b_{ij} \in \{-1, 0, 1\}$, for which the solution has been derived above.

Trait Model

The **B** matrix in the trait model is characterized by constant vector length irrespective of orientation, thus the effects of loci per trait depend on the angles of these vectors in phenotypic space:

$$\mathbf{B} = \begin{pmatrix} \frac{\sin\theta}{|\mathbf{b}_1|} & \frac{\sin(\theta+x)}{|\mathbf{b}_2|} \\ \frac{\cos\theta}{|\mathbf{b}_1|} & \frac{\cos(\theta+x)}{|\mathbf{b}_2|} \end{pmatrix},$$

where $|\mathbf{b}_j|$ is the length of the substitution-effect vector at *j*th locus, which we assume in the following to be unit length in all vectors; θ is the angle of the first vector from some reference vector (e.g., trait axis 1), and x is the angle between the two vectors, so that $-\pi < x < \pi$. Average conditional evolvability for a two-gene system is then

$$\bar{c} = Det[\mathbf{B}] = \sin\theta\cos(\theta+x) - \cos\theta\sin(\theta+x) = \sin x.$$

The solution for this system is obvious: the average conditional evolvability is maximized when $x = \pm\pi/2$, and it is irrelevant how the two vectors are oriented with respect to the trait axes (θ cancels out in the equation). It follows that the degree of pleiotropy is irrelevant in this model, as long as the two vectors are orthogonal.

Extending the model to multiple genes, we again use the observation that the squared average conditional evolvability is the sum of the squared determinants of all possible two-gene sub-matrices. In terms of between-vector angles, this means that average conditional evolvability equals the square root of the sum of the squared sinus function of angles between all possible pairs of locus vectors. Note that the initial reference angle θ always cancels out, meaning that the orientation of the genetic effect vectors relative to the phenotypic axes is irrelevant. This also means that the degree of pleiotropy is not the essential criterion for optimization.

Due to rotational invariance in the trait model, we can expect different equally optimal solutions when optimizing $n(n-1)/2$ angles simultaneously. To characterize these solutions we consider that, because in the trait model the length of the effect vector is not affected by its orientation relative to the trait axes, the total genetic variance (evolvability) is constant. In terms of the **G** matrix this means that the trace of the matrix ($\text{trace}[\mathbf{G}] = \lambda_1 + \lambda_2$) is constant. For the two-trait system $\bar{c} = \sqrt{\lambda_1\lambda_2}$, which is maximized when $\lambda_1 = \lambda_2 = \text{trace}[\mathbf{G}]/2$, and therefore when the genetic covariance of **G** equals zero.

Note that the maximum is at $\max \bar{c} = \frac{\text{trace}[\mathbf{G}]}{2} = \frac{G_{11}+G_{22}}{2} = \bar{e}$.

Multiple equally optimal solutions for a **B** matrix fulfill this condition (Fig. 2b). The number of trigonometric variables representing vectors in **B** precludes defining an exhaustive set of solutions. Given the vectors of equal length, the set of solutions includes e.g., all **B** matrices in which each vector has an orthogonal counterpart, however the way such pairs of vectors are arranged relative to other pairs is arbitrary. Complete modularity is a special case of this vector distribution, in which all pairs of vectors point into two identical directions, forming two orthogonal bundles. Further distributions of vectors for which $\bar{c} = \bar{e}$ are those with equally spaced vectors ("Equally-spaced vector arrangement"; Fig. 2b), or equally spaced bundles of vectors, etc.

The condition $G_{12} = 0$ does not specify how the single covariance contributions add up. In general, all solutions where negative and positive covariance of loci cancels out are equally optimal as those where no covariance is generated. In reality the vectors can be of different lengths, contributing differently to (co)variance. The equilibrium is then reached not by the equal numbers of vectors generating positive and negative variance, but by balancing their contributions. Thus, the amount of variance allocated at certain angles is also relevant.

Appendix 2

Here we show that a modular vector arrangement and an equally spaced vector arrangement yield maximal average conditional evolvability in the trait model.

Modular Arrangement

The average conditional evolvability can be calculated for a general model with orthogonal bundles of vectors by grouping the between-vector angles into three groups: between vectors within the bundle 1, between vectors within the bundle 2, and the angles between vectors of the opposite bundles. The sine of angles of the first two groups equal 0, and the sine of the between-group angles all equal 1. Hence:

$$\bar{c}^2 = 2\frac{n}{4}\left(\frac{n}{2}-1\right)\sin^2 0 + \left(\frac{n}{2}\right)^2 \sin^2\frac{\pi}{2} = \left(\frac{n}{2}\right)^2 \Rightarrow \bar{c} = \frac{n}{2}.$$

This is the same evolvability for the fully modular GP map as in the character model. Note that all vectors contribute the same amount of variance. In a case of the modular two-trait system $n/2 = \bar{e}$.

Equally-Spaced Vector Arrangement

In this case the **B** matrix has a uniform distribution of vectors around 2π (the angles between neighboring vectors are equal). Let n be the number of vectors (angles), then each angle between neighbor vectors is $2\pi/n$. The squared average conditional evolvability (=the sum of the squared sine between all vector-pairs) is then

$$\begin{aligned}\bar{c}^2 &= (n-1)\sin^2\frac{2\pi}{n} + (n-2)\sin^2 2\frac{2\pi}{n} + \cdots \\ &\quad + (n-(n-1))\sin^2\left((n-1)\frac{2\pi}{n}\right) \\ &= \sum_{j=1}^{n-1}(n-j)\sin^2\left(j\frac{2\pi}{n}\right) \\ &= \sum_{j=1}^{n-1}\frac{1}{2}(n-j)\left(1-\cos\left(2j\frac{2\pi}{n}\right)\right) \\ &= \frac{1}{2}\left[\frac{n(n-1)}{2}+\frac{n}{2}\right] \\ &= \frac{n^2}{4},\end{aligned}$$

and therefore, $\bar{c} = n/2 = \bar{e}$. The last is true because the **B** matrix is composed of n columns each with two elements, a sine and a cosine of the same angle α. The sum of their squares is always one, and the average evolvability equals half the sum across n such columns.

Appendix 3

Here we show that the trait and character models of pleiotropy are equivalent at full pleiotropy, i.e., when in a trait model, $\theta = s(\pi/4)$, so that s $\in\{1, 2,\ldots,8\}$).

House-of-Cards Approximation

Considering the contribution of a single locus j to the genetic variance of a character 1. In the character model, the jth **B** matrix column is $\mathbf{b}_j = \begin{pmatrix}\pm 1\\ \pm 1\end{pmatrix}$, contributing the following variance to the character 1: $G_{11j} = \frac{4u}{s_1+s_2}$. In the trait model, the corresponding column is $\mathbf{b}_j = \begin{pmatrix}\pm\cos\theta\\ \pm\sin\theta\end{pmatrix}$, contributing the following amount of variance to the character 1: $G_{11j} = \frac{4u\cos^2\theta}{s_1\cos^2\theta + s_2\sin^2\theta}$ (where θ is the angle between the mutational effect vector and the axis of character 1; see Fig. 1).

Gaussian Approximation

In the character model, with locus-vector $\mathbf{b}_j = \begin{pmatrix}\pm 1\\ \pm 1\end{pmatrix}$, the variance contribution to the character 1 is $G_{11j} = \frac{\sigma_m}{\sqrt{s_1+s_2}}$; whereas in the trait model of pleiotropy, the locus vector $\mathbf{b}_j = \begin{pmatrix}\pm\cos\theta\\ \pm\sin\theta\end{pmatrix}$ contributes $G_{11j} = \frac{\sigma_m\cos^2\theta}{\sqrt{s_1\cos^2\theta + s_2\sin^2\theta}}$ to the variance of the trait 1. These contributions are equivalent when $\theta = \pi/4$.

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