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Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability

Thomas F. Hansen*

Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA

Abstract

Evolvability is the ability to respond to a selective challenge. This requires the capacity to produce the right kind of variation for selection to act upon. To understand evolvability we therefore need to understand the variational properties of biological organisms. Modularity is a variational property, which has been linked to evolvability. If different characters are able to vary independently, selection will be able to optimize each character separately without interference. But although modularity seems like a good design principle for an evolvable organism, it does not therefore follow that it is the only design that can achieve evolvability. In this essay I analyze the effects of modularity and, more generally, pleiotropy on evolvability. Although, pleiotropy causes interference between the adaptation of different characters, it also increases the variational potential of those characters. The most evolvable genetic architectures may often be those with an intermediate level of integration among characters, and in particular those where pleiotropic effects are variable and able to compensate for each other's constraints.

Keywords: Evolvability; Pleiotropy; Modularity; Genetic constraint; Eye evolution; Adaptation; Adaptability; Variability; Genetic variance

1. Introduction

One of the most fundamental, yet puzzling, properties of living organisms is their amazing ability to evolve. In mainstream evolutionary theory this evolvability has been taken almost for granted. Most studies of adaptation simply assume that the organism is variationally capable of producing the character states that lead to the optimal phenotype. Within certain selective constraints, such as those stemming from limited time and energy budgets, all possible variants of defined characters are usually assumed to be available for selection.

Nowhere is this standard set of variational assumptions better illustrated than in Nilsson and Pelger's (1994) "A pessimistic estimate of the time required for an eye to evolve". With this elegant simulation model, Nilsson and Pelger show how a complex eye can evolve in a surprisingly short amount of time under selection for improved visual acuity. Starting with a sheet of photoreceptors sandwiched between a transparent and a pigment layer of tissue, the eye evolves first by changes in the size and shape of the tissue layers to form a pinhole eye. The refractive index of the vitreous body then change at the aperture to form a graded-index lens. This model assumes that continuous genetic variation arises in a number of defined traits describing the size, shape and optical properties of the involved tissues. It assumes that the variation in each trait is independent of the variation in the other

* Tel.: +1-850-644-4779.
E-mail address: thomas.hansen@bio.fsu.edu (T.F. Hansen).

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traits, and of the rest of the organism. In essence, it assumes that variation along the entire continuum from the initial sheet of photoreceptors to the final complex eye is made readily available for selection on a step-by-step basis. In fact, as Nilsson and Pelger point out, this makes the model comparable to the evolution of a one-dimensional quantitative character with unlimited variability (i.e. unlimited capability to produce more extreme variants). We may ask how pessimistic it really is to assume that an organ as complex as an eye should have a variational basis that is no more complex than that of simple univariate size trait?

Still, such assumptions may not be unwarranted. Complex eyes have indeed evolved, rates of microevolution can be very high (Hendry and Kinnison, 1999), and the success of optimality analyses of adaptation speaks to astonishing variational potential in many characters. An adaptationist may simply take the variability as an empirical fact that justifies the focus on selection per se. A skeptic may argue that the optimality is usually more of an assumption than a hypothesis (Mitchell and Valone, 1990), that stasis is more common than rapid change (Williams, 1992), and that the more advanced types of eyes have evolved only a few times and are far from perfect (Dawkins, 1996). Both the skeptic and the adaptationist should agree, however, that a better understanding of the variational capabilities of biological systems is needed. To have a complete theory of evolution, the adaptationist needs to explain why organisms are variationally able to respond so easily to selective pressures, and the skeptic needs a variational theory to understand and predict constraints on evolutionary change.

The recent surge of interest in the problem of variation and its implications for evolvability is therefore an important development in evolutionary theory (Wagner and Altenberg, 1996; Von Dassow and Munro, 1999; Rutherford, 2000; Stern, 2000). Evolvability has been a central concern in several recent books by authors with rather different backgrounds and perspectives (Conrad, 1983; Kauffman, 1993; Dennett, 1995; Maynard Smith and Szathmáry, 1995; Dawkins, 1996; Raff, 1996; Gerhart and Kirschner, 1997), and many principles of evolvability have been suggested. These principles include duplication and divergence, robustness, dissociability, modularity, symmetry, redundancy, co-option, recombination, cryptic variation, “evolutionary cranes”, “extradimensional bypass”, “the edge of chaos”, and the emergence of new hierarchical levels of organization.

In this essay I will focus on the concept of modularity, which is widely seen as one of the central principles of evolvability. The idea is that modular organization favors evolvability by allowing one module to change without interfering with the rest of the organism (e.g. Riedl, 1977; Wagner, 1996). Fisher (1958) demonstrated that the probability of a random mutation being favorable was a steeply decreasing function of the number of traits it affected. Simultaneous random changes in many parts of a highly integrated structure are not likely to improve its function, as the chance improvement of one part will almost always be swamped by deleterious effects in many other parts. But if the parts are variationally independent, selection gets the chance to tune them one at a time, thereby improving the probability of finding improvements.

The importance of modularity, or some similar concept, for evolvability has been recognized for a long time (e.g. Needham, 1933; Olson and Miller, 1958; Berg, 1960; Riedl, 1977, 1978; Bonner, 1988), and modular thinking is deeply embedded in biology. Lewontin (1978) pointed out that the entire adaptationistic program assumes that characters have what he termed quasi-independent evolutionary potential. In fact, as reviewed in Wagner (2001), the concept of a biological character presupposes a degree of modularity and usually carries the implicit assumption that characters can evolve independently of each other. Returning to Nilsson and Pelger’s analysis of the evolution of the eye, we can identify one source of the immense evolvability of their model as stemming from the division of the eye into a set of characters that are variationally independent both of each other and of the rest of the organism. This is a seemingly innocent, but perhaps very optimistic assumption.

Indeed, the eye characters in the model are unlikely to be modular in a strict sense. To illustrate this point, consider a particular character, the lens. Although the lens can be recognized as a distinct developmental module, it has a number of interrelationships with other characters. In amphibians, the lens is formed through a series of inductive interactions with other tissues such as the retina and even heart mesoderm (Raff, 1996). The defining feature of the lens, its

elevated refractive index, is controlled by the secretion of large amounts of soluble crystallines into the cell cytoplasm. The crystallines are not strongly specialized for this role. Rather, they are functional proteins of varied origin that often have a dual enzymatic function in other cells (Wistow, 1993; Raff, 1996). Thus, the refraction of the lens is not automatically variationally independent of other organismal functions. The recruitment or co-option of lens crystallines may have been constrained by the need to preserve other functions of the recruited proteins. Although, Nilsson and Pelger may have made conservative assumptions about the amount of genetic variation in this trait, they were not conservative in assuming that the variation was unconstrained by pleiotropic links to other aspects of the organism. Whether such pleiotropy may have constrained the evolution of the lens is unknown, and this is of central importance to assessing evolvability.

A link between modularity and evolvability has also emerged in evolutionary computer science (Wagner and Altenberg, 1996). Indeed, modularity is a basic principle of good programming. Engineers have learned to design robust and flexible programs by dividing the task up into a set of modules, subroutines or objects, with simple well-defined interfaces. Then each module can be designed in isolation. The intuitive appeal of modularity may stem from this engineering analogy. It is a powerful and easily understandable principle, which appeals to our engineering minds and to our instinct for organizing the world into separate objects.

But remember Jacob's (1982) metaphor: natural selection is not an engineer. Evolution does not operate from simple and elegant blueprints of the most efficient solution to a given problem. Rather it operates like a "tinkerer", building shortsighted ad hoc solutions with whatever materials happen to be available. Modularity may indeed be a simple, logical and efficient way of achieving evolvability, but it does not therefore follow that it is the biological basis of evolvability. Nor does the logic of modularity exclude the possibility that evolvability is achieved in ways that appear complex and illogical to our minds. In this essay I present some formal analyses of how modularity and pleiotropy relates to evolvability, and ask whether modularity is the only, or even the most efficient, way of structuring an evolvable genetic architecture.

2. Evolution of modularity: integration and dissociation

Modularity enhances evolvability by allowing characters to evolve without interference, but modularity may also hamper evolvability by reducing the number of genes that can affect the character; thereby reducing its mutational target size. The most likely way in which modularity may evolve is by removing pleiotropic effects among characters (Wagner, 1996; Wagner and Altenberg, 1996). It is clear that this will reduce the variability of the character from which the effect is removed and, as I will show below, a pleiotropic effect is only an absolute constraint if the characters are perfectly correlated. Thus, removing a pleiotropic effect may increase the evolvability of one character and reduce the evolvability of another. This means that it is not clear whether the process of dissociating two characters by removing pleiotropic effects will enhance or diminish evolvability of the system as a whole.

From a different perspective, it is clear that recruiting more genes to affect a character can increase its evolvability. Genes available for recruitment will typically already have effects on other characters, and the evolution of evolvability by recruitment of genes will therefore, at least initially, be associated with the evolution of increased integration among characters. Consider a character under directional selection. An allele that introduces a novel effect on this character may be picked up by selection and increase in frequency. This will lead to compensatory changes in the other characters affected by this gene, and eventually the new allele may go to fixation. If the new effect was acquired through the appearance of a new enhancer that expresses the gene on the character under directional selection, then almost all subsequent mutations of this gene will inherit this pleiotropic effect. Thus, through integration, the character has acquired a new source of mutational variability, which makes it more evolvable.

The recruitment of crystallines to the lens is a potential example of such a process. Eventually, gene duplication may allow specialization and the pleiotropic constraints can be removed, but it does not seem particularly likely for a duplication to be the first step in the process. In the case of crystallines, some appear to have duplicated and some do not (Wistow, 1993; Raff, 1996). Nevertheless these examples demonstrate that the evolution of modularity does not imply the

evolution of evolvability, and that the evolution of evolvability does not imply the evolution of modularity.

3. Quantifying character evolvability as independent evolutionary potential

3.1. Conditional evolvability

Consider two characters that spend most of their time under stabilizing selection, but occasionally experience episodes of directional selection when their selective regimes are changing. We assume that the selective regimes of the two characters are independent in the sense that the episodes of directional selection they experience are uncorrelated. The identification of characters may be seen as a choice of coordinate system for the morphology. We are thus choosing a coordinate system where the bases are independent with respect to changes in selective regime. Pleiotropy is thus defined relative to this coordinate system.

In this context, the relevant evolvability of a character is its ability to respond to directional selection when the other character is under stabilizing selection, as in the corridor models of Wagner (1984, 1988) and Bürger (1986). We refer to this as the conditional evolvability of the character, and the purpose of the following model, first presented in Hansen et al. (2003a), is to derive an operational measure of the conditional evolvability.

Let $\boldsymbol{y}$ designate the (vector) character under directional selection with selection gradient β, and let $\boldsymbol{x}$ designate the (vector) character under stabilizing selection with quadratic fitness function $w_0 - \boldsymbol{x}'\boldsymbol{S}\boldsymbol{x}$. Here, w_0 is a constant describing maximum fitness when $\boldsymbol{x}$ is at its optimum (at $\boldsymbol{x} = \boldsymbol{0}$), $\boldsymbol{S}$ is a matrix of selection parameters that describe the decay in fitness as $\boldsymbol{x}$ is displaced from the optimum, and $'$ denotes transpose. Let capital letters, $\boldsymbol{Y}$ and $\boldsymbol{X}$, denote the population averages of the characters. Unless the characters are uncorrelated, directional selection on $\boldsymbol{y}$ will lead to a correlated response in $\boldsymbol{X}$. This means that $\boldsymbol{X}$ will become displaced from its optimum and there will appear a selection pressure to bring $\boldsymbol{X}$ back towards the optimum. This selection pressure will then constrain the further response in $\boldsymbol{Y}$. In this situation we describe selection on $\boldsymbol{x}$ with a local linear approximation of the fitness function around the mean vector $\boldsymbol{X}$. This selection gradient is $\beta_{\boldsymbol{x}} = -(\boldsymbol{S} + \boldsymbol{S}')\boldsymbol{X}$. With this in hand, we can use Lande's (1979) equations for the response to directional selection

$$\Delta\boldsymbol{Y} = \boldsymbol{G}_{\boldsymbol{y}}\beta_{\boldsymbol{y}} - \boldsymbol{G}_{\boldsymbol{yx}}(\boldsymbol{S} + \boldsymbol{S}')\boldsymbol{X} \tag{1}$$

$$\Delta\boldsymbol{X} = \boldsymbol{G}'_{\boldsymbol{yx}}\beta_{\boldsymbol{y}} - \boldsymbol{G}_{\boldsymbol{x}}(\boldsymbol{S} + \boldsymbol{S}')\boldsymbol{X}$$

where $\boldsymbol{G}_{\boldsymbol{y}}$ and $\boldsymbol{G}_{\boldsymbol{x}}$ are the additive genetic variance matrices of $\boldsymbol{y}$ and $\boldsymbol{x}$ and $\boldsymbol{G}_{\boldsymbol{yx}}$ is their additive genetic covariance matrix. Now, if the genetic variances and covariances remain constant, $\boldsymbol{X}$ will reach an equilibrium displacement from its optimum at the value

$$\boldsymbol{X}^* = (\boldsymbol{S} + \boldsymbol{S}')^{-1}\boldsymbol{G}_{\boldsymbol{x}}^{-1}\boldsymbol{G}'_{\boldsymbol{yx}}\beta_{\boldsymbol{y}} \tag{2}$$

If this equilibrium displacement value is fed back into the expression for the response in $\boldsymbol{Y}$, we get

$$\Delta\boldsymbol{Y} = (\boldsymbol{G}_{\boldsymbol{y}} - \boldsymbol{G}_{\boldsymbol{yx}}\boldsymbol{G}_{\boldsymbol{x}}^{-1}\boldsymbol{G}'_{\boldsymbol{yx}})\beta_{\boldsymbol{y}} \equiv \boldsymbol{G}_{\boldsymbol{y}|\boldsymbol{x}}\beta_{\boldsymbol{y}} \tag{3}$$

Thus, when the selective forces that act on $\boldsymbol{x}$ have come to an equilibrium, the continued response in $\boldsymbol{Y}$ is determined by the conditional genetic variance matrix, $\boldsymbol{G}_{\boldsymbol{y}|\boldsymbol{x}}$. This entity is simply a measure of the genetic variation in the residuals from a regression of the genetic value of $\boldsymbol{y}$ on the genetic value of $\boldsymbol{x}$. Thus, the asymptotic evolvability of $\boldsymbol{y}$ is determined by the genetic variation that remains when $\boldsymbol{x}$ is held genetically fixed. The conditional genetic variance is thus a theoretical measure of the short-term conditional evolvability of a character as long as the pattern of genetic variances and covariances remain constant.

This is a heuristic result. The $\boldsymbol{G}$-matrix may change not only due to changes in underlying gene frequencies and coupling disequilibrium, but also due to epistatic interactions among genes (Hansen and Wagner, 2001). Furthermore, the strength of selection is unlikely to remain constant over sufficiently long periods to reach the asymptotic response. The conditional variance matrix may still capture the qualitative dynamics of mosaic evolution and serve as a meaningful theoretical parameterization of evolvability.

The concept of conditional evolvability is thus a tool for quantifying the independent evolutionary potential of a character, and may thus help operationalize Lewontin's (1978) notion of quasi-independence as a prerequisite for adaptation.

In the following we will study the effects of character integration by using the conditional $\boldsymbol{G}$-matrix as a

proxy for evolvability. It is then useful to factorize the conditional $\boldsymbol{G}$-matrix into the genetic variance and a coefficient of determination

$$\boldsymbol{G}_{y|x} = \boldsymbol{G}_y(\boldsymbol{I} - \rho^2_{yx}) \tag{4}$$

where $\boldsymbol{I}$ is the identity matrix, and $\rho^2_{yx} = \boldsymbol{G}_y^{-1}\boldsymbol{G}_{yx}\boldsymbol{G}_x^{-1}\boldsymbol{G}'_{yx}$ is the squared multivariate correlation coefficient. In the next section we will, however, focus on univariate characters where ρ_{yx} is simply the genetic correlation between **y** and **x**.

3.2. Optimizing evolvability

Many changes in genetic architecture will have alternate effects on the evolvability of different characters. If a pleiotropic link is added this will reduce the evolvability of those characters that were previously affected by the gene and increase the evolvability of those characters that were not previously affected. Thus, a general measure of the evolvability of a set of characters as a whole is the sum of their conditional evolvabilities, where each character is conditioned on all the others. As a simplification we consider a phenotype consisting of only two univariate characters x and y, so that the evolvability can be measured as

$$E = \boldsymbol{G}_{\mathbf{y}|\mathbf{x}} + \boldsymbol{G}_{\mathbf{x}|\mathbf{y}} = (\boldsymbol{G}_{\mathbf{y}} + \boldsymbol{G}_{\mathbf{x}})(1 - \rho^2_{\mathbf{yx}}) \tag{5}$$

Consider first a simple model where we assume that there are three underlying sources of variation: one $\sigma^2_{\mathbf{y}}$ that affects only **y**, one $\sigma^2_{\mathbf{x}}$ that affects only **x**, and one $\sigma_{\mathbf{yx}}$ that affects them both. We assume that the sum of these three variance components is a constant, σ^2_{T}. Thus, genes can have effects either on **y** alone, on **x** alone or on both equally. The question now is what arrangement of genes among these possibilities will optimize the evolvability as given by E. To answer this, we compute the following relations:

$$\boldsymbol{G}_{\mathbf{y}} = \sigma^2_{\mathbf{y}} + \sigma_{\mathbf{yx}}, \quad \boldsymbol{G}_{\mathbf{x}} = \sigma^2_{\mathbf{x}} + \sigma_{\mathbf{yx}}, \quad G_{\mathbf{yx}} = \sigma_{\mathbf{yx}} \tag{6}$$

From this we find that the evolvability is given as

$$E = \sigma^2_{\mathbf{y}} + \sigma^2_{\mathbf{x}} + \sigma^2_{\mathbf{y}}\left(\frac{\sigma^2_{\mathrm{T}} - \sigma^2_{\mathbf{y}} - \sigma^2_{\mathbf{x}}}{\sigma^2_{\mathrm{T}} - \sigma^2_{\mathbf{x}}}\right) + \sigma^2_{\mathbf{x}}\left(\frac{\sigma^2_{\mathrm{T}} - \sigma^2_{\mathbf{y}} - \sigma^2_{\mathbf{x}}}{\sigma^2_{\mathrm{T}} - \sigma^2_{\mathbf{y}}}\right) \tag{7}$$

It is straightforward to show that this function is maximized when

$$\sigma^2_{\mathbf{y}} = \sigma^2_{\mathbf{x}} = \sigma^2_{\mathrm{T}}\frac{(\sqrt{3}-1)}{\sqrt{3}}, \qquad \sigma_{\mathbf{yx}} = \sigma^2_{\mathrm{T}}\frac{(2-\sqrt{3})}{\sqrt{3}} \tag{8}$$

Thus, the evolvability is maximized when approximately 16% of the genetic variation is pleiotropic, 42% is confined to character **y** and 42% to character **x**.

Why is evolvability maximized at an intermediate level of genetic integration? Consider first the situation where the two characters are uncorrelated. Then the introduction of pleiotropic effects will increase the genetic variation of the characters and this has little cost, as there is ample genetic variation in both characters that can compensate for the correlated changes. However, as the correlation increases, the genetic architecture becomes less and less able to compensate for the correlated changes. Eventually, the addition of further pleiotropic effects will decrease the evolvability. In the limit as the characters become completely correlated the evolvability drops to zero. There is no variation that can be used for compensatory changes, and the characters are unable to evolve independently.

3.3. Hypothesis: evolvability is maximized by variable pleiotropic effects

The above model included only a single type of positive pleiotropy where each allele had the same effect on the two characters. However, antagonistic pleiotropic effects, where an allele increases the effect of one character and decrease the effect of another, are also possible. Antagonistic pleiotropy may for example be implemented through genes that control the allocation of resources to the characters. Genes with different types of pleiotropic effects may be able to compensate for each other's constraint, and we expect a genetic architecture with a range of different pleiotropic interactions to be more evolvable than one where all genes have similar effects.

In fact, the genetic architectures that maximize E, as given by (5), are those where all genes have pleiotropic effects, but these are what Baatz and Wagner (1997) called "hidden pleiotropic effects" where positive and antagonistic pleiotropy cancel. In this situation, all

genes affect both characters, thus maximizing $\boldsymbol{G}_y$ and $\boldsymbol{G}_x$, while the positive and negative genetic covariances created by alternate pleiotropic effects cancel, making ρ_{yx} equal to zero. Thus, in this model, the most evolvable genetic architectures are those where all genes affect both characters, but they do so in maximally different ways.

Variation in pleiotropic effects may be even more important in facilitating the evolvability of suites of characters. The above model focused on the independent evolvability of single traits, i.e. along two particular directions in morphospace, but adaptation to environmental change will typically require the adjustment of several traits in specific combinations that will differ from time to time. The most versatile genetic architecture may very well be the one with maximal variation in pleiotropic effects. This will allow selection of alleles with just the right suite of effects, and also make a full range of compensatory changes available.

4. Pleiotropy and variation

4.1. Pleiotropy and the maintenance of variation under stabilizing selection

A significant negative effect of pleiotropy on short-term evolvability is that it increases the strength of stabilizing selection acting on individual loci, thereby reducing the amount of variation maintained at a locus under a balance between stabilizing selection and mutation (Lande, 1980; Turelli, 1985; Wagner, 1989a). This reduction in standing genetic variation reduces the amount of variation available for response to directional selection when the environment changes. The increase in the strength of selection on individual loci must, however, be weighted against the increased number of loci that affects the individual characters. The analyses of Turelli (1985) and Wagner (1989a) show that the effects of pleiotropy on variance maintained are complicated and model dependent.

For the house-of-cards approximation, which is valid when mutations are rare and selection not too weak on individual loci, there seem to be no general tendency for pleiotropy to reduce the amount of variation maintained in individual traits. In the special case where all mutation rates, pleiotropic effects and selection strengths are the same, the variance maintained in a character affected by n loci that each have pleiotropic effects on m traits is given by

$$\text{variance} = \frac{4nu}{sm} \tag{9}$$

where s is strength of stabilizing selection on each character (= eigenvalue of $\boldsymbol{S}$), and u is per-locus mutation rate (Wagner, 1989a). The same scaling with respect to m also holds if pleiotropic effects vary, but cancel on average, i.e. they are hidden (Turelli, 1985; Bürger, 2000). This shows that the variation maintained at a locus is $1/m$ times the amount maintained if there are no pleiotropic effects, but since the average number of loci affecting a trait, n, must be proportional to the average number of traits affected by each locus, m, there are no obvious scaling of genetic variation with pleiotropy in the house-of-cards model.

With the Gaussian approximation, which is valid when mutation rates are high and selection weak on individual loci, there may actually be a tendency for standing genetic variation in individual characters to increase with integration. The reason is that in the Gaussian equilibrium the variance scales with the square root of selection strength, making us expect the variance to scale with $1/\sqrt{m}$ if the pleiotropic effects are uniform (as in Wagner, 1988), and since the variance is still proportional to n, the average trait variance may increase. Also, when the pleiotropic effects are random and hidden, the amount of variation maintained in a single character is unaffected by pleiotropy in the Gaussian model (Bürger, 2000), and will thus increase with the number of loci affecting the trait.

Thus, although the effects of pleiotropy on variation maintained under mutation-selection balance depend on the genetic details, there are no general mechanisms that make integration reduce the amount of variation maintained in individual traits; when the Gaussian approximation is valid there may even be a tendency for individual traits to exhibit more variation when they are integrated with other traits. The effects on evolvability, however, also depend on how much genetic correlation is created. If pleiotropic effects are variable and largely hidden, the evolvability may increase, if pleiotropic effects are largely uniform, evolvability may decrease.

4.2. Costs to pleiotropy

Baatz and Wagner (1997) found that the response to directional selection could be slowed down by hidden pleiotropic effects with a character under stabilizing selection. This contrasts with the model above and may be due to several factors. One of these is the effect of stabilizing selection on variance. Clearly, stabilizing selection on pleiotropic effects reduces the evolvability of a character if gene number is kept constant, but as argued above this does not mean that a pleiotropic genetic architecture necessarily exhibit less genetic variation in mutation-selection equilibrium and therefore is less evolvable. Another effect is induced apparent stabilizing selection on the directionally selected character. Concavity of the fitness function induce a term, $-\mathrm{sCov}[\mathbf{y}, \mathbf{x}^2]$, to the selection response in Eq. (3) (Turelli, 1988). With strong stabilizing selection, this may constitute a substantial cost to pleiotropy, and with non-Gaussian allelic distributions, it may also create a cost of hidden pleiotropic effects.

Another important short-term cost that applies to genetic architectures with nonzero genetic correlation is the load that is created when correlated selection displaces characters from their optima (see Eq. (2)). This selection load is independent of the strength of selection on the characters, and temporary in the sense that it will disappear when directional selection is relaxed. Thus, the enhanced evolvability resulting from more pleiotropy will usually pay the price of an increased selection load. Notice, however, that this is a cost of genetic correlation and not of pleiotropy per se.

5. Evolvability on longer time scales

5.1. Pleiotropy and the rate of advantageous mutation

Fisher's (1958) geometric model of adaptation shows that the probability of a random mutation to be advantageous is a decreasing function of the number of traits it affects (see also Kimura, 1983; Hartl and Taubes, 1998; Orr, 1998). Fisher's model was a geometric attempt at capturing the notion that if many simultaneous random changes were made to a complex apparatus, like a microscope, this would be extremely unlikely to improve the apparatus. This is a devastating argument against the evolvability of complex integrated genetic architectures, and a strong argument in favor of modularity, as random changes to isolated parts have a much higher probability of improving function. But again, we need to weigh these considerations against the fact that integrated genetic architectures provide for more variability in any one part. To do this we analyze a model similar to the one presented by Fisher, but from the perspective of the evolvability of a single character that is displaced from its optimum as above.

Consider a univariate character under directional selection with selection gradient β. Mutations affecting this character are constrained by pleiotropic effects on m other univariate characters under independent stabilizing selection. The effect on fitness of a mutation with effect $\mathbf{y} > 0$ on the first character and x_i on the ith constraining character is then

$$\beta\mathbf{y} - \sum_i s_i x_i^2 \tag{10}$$

where s_i is the strength of stabilizing selection on the ith character and the sum is over the m constraining characters. For this mutation to be advantageous (10) has to be positive. Now assume that the pleiotropic effects, x_i, of new mutations are normally distributed with variance σ^2, and that $s_i = s$ for all i. Then we can write (10) as

$$\beta\mathbf{y} - s\sigma^2\chi^2(m) \tag{11}$$

where $\chi^2(m)$ is a chi-square random variable with m degrees of freedom. Thus, the mutation is advantageous whenever $\chi^2(m) < r \equiv \beta\mathbf{y}/s\sigma^2$; the parameter r is a measure of the relative strength of directional versus stabilizing selection. Clearly, the probability of being advantageous is a decreasing function of m, but if we assume that the number of potentially advantageous mutations affecting the directionally selected character is proportional to the level of pleiotropy, m, then we find that the rate of appearance of advantageous mutations will actually be elevated by pleiotropy whenever

$$(1 + m)\,\mathrm{Prob}[\chi^2(m) < r] > 1 \tag{12}$$

This shows that pleiotropy will increase the advantageous mutation rate provided directional selection is sufficiently strong relative to stabilizing selection on

the constraining characters. To get at the rate of evolution in this model, however, we also need to consider the effects of pleiotropy on the expected selective advantage of the mutations (Kimura, 1983). The rate of evolution is the advantageous mutation rate multiplied with the fixation probability of those mutations. The fixation probability is approximately twice the expected selective advantage of those mutations. Then the rate of evolution becomes

$$2\beta \mathbf{y} u(1+m)\,\mathrm{Prob}[\chi^2(m) < r] \times \left(1 - \frac{E[\chi^2(m)|\chi^2(m) < r]}{r}\right) \quad (13)$$

where u is the total mutation rate. It can be shown by integration that

$$\mathrm{Prob}[\chi^2(m) < r] = 1 - \frac{\Gamma[m/2, r/2]}{\Gamma[m/2]} \quad (14a)$$

$$E[\chi^2(m)|\chi^2(m) < r] = \frac{m\Gamma[m/2] - 2\Gamma[1+m/2, r/2]}{\Gamma[m/2] - \Gamma[m/2, r/2]} \quad (14b)$$

where $\Gamma[a]$ is the gamma function and $\Gamma[a, b]$ the incomplete gamma function. Using the case without pleiotropy as a benchmark, we can now compute some examples. If there are pleiotropic effects on one other character, $m = 1$, we find that that the rate of appearance of advantageous mutations is elevated provided $r > 0.46$, and the rate of evolution is elevated when $r > 1.08$. These criteria are more likely to be fulfilled for mutations with small effects, since it is reasonable to assume that σ^2 scales with $\mathbf{y}^2$. Thus, if there is strong directional selection, the addition of a pleiotropic effect will enhance evolvability, as long as the mutational effects are not too large or stabilizing selection too strong. As more pleiotropic effects are added, the criteria become more difficult to fulfill. With $m = 3$, the criteria are $r > 1.21$ and $r > 2.44$, and with $m = 100$, they become $r > 70.0$ and $r > 84.7$, respectively. Thus, for a given strength of directional versus stabilizing selection (i.e. for a given r), evolvability may be maximized at an intermediate level of pleiotropy.

In conclusion, a certain amount of integration is likely to enhance evolvability of a character that is very maladapted, but as the character is becoming better adapted, the pleiotropy may become a constraint.

5.2. Short-term pessimism or long-term optimism?

The pessimism in Nilsson and Pelger's (1994) argument refers to the conservative assumptions made about the per generation evolvability of the eye. If we think of the eye as a single univariate character evolving towards the final stage, we may characterize the evolvability of this character by observing that the assumptions imply that the additive genetic variation scaled by the squared trait mean was 0.01%. As detailed in Hansen et al. (2003b), this is a measure of evolvability that can be interpreted as the % response per generation to directional selection that is as strong as selection on fitness itself. Thus, even with this strong selection, the response in the character would only be a hundred of a percent per generation. Nilsson and Pelger in fact assumed a moderately strong causal link to fitness (their assumptions imply that the elasticity of fitness with respect to trait was 0.5) to obtain an expected response per generation of 0.005%. Still, these are conservative assumptions from the quantitative genetics perspective, and particularly so with respect to variational properties.

Conservative, however, only from the perspective of the short-term evolvability of an isolated character. An evolvability of 0.01% is less conservative when it is realized that the measure should only include the additive variation that remains after conditioning on other characters. How much the evolvability of different characters is reduced by pleiotropic constraints is an important empirical question in need of research. Another crucial assumption is that the genetic variance remains constant as the character is changing. If evolvability over more than a few dozen generations or so is at issue, the relevant variational property is not the standing genetic variation, which will get exhausted, but rather the ability to produce new genetic variation through mutation. The amount of variation introduced each generation by mutation is very variable across traits, but may often be on the order of 100 to 1000 part of standing genetic variation (Lynch, 1988; Houle et al., 1996; Houle, 1998; Lynch et al., 1999). If the mutational variation is seen as the rate limiting process, a per generation evolvability of 0.01% appears more optimistic.

This is particularly so as the estimates of novel mutational variation still assumes that the character is unconstrained from other characters. The relevant

mutational evolvability is given by the conditional mutational variance matrix analogous to the conditional $\boldsymbol{G}$-matrix in (3). The properties of mutational variance matrices are very poorly known, and it is hard to assess the level of constraint on the mutational variance. One would, however, expect mutational variation to be more seriously affected by deleterious pleiotropic side effects than what is the case for segregating variation, as the latter is already filtered by selection. There is a need for estimates of "non-deleterious" mutation rates.

Another optimistic aspect of the eye model is the assumption of a continuous path of improvement that allows the eye to evolve as if it was a single character. This is equivalent to the assumption of a smooth adaptive landscape. One could argue that what the model in fact shows is that a smooth path in the fitness landscape can exist (Dawkins, 1996). But note that this is then conditional on the particular variational assumptions that were made. The landscape may be smooth relative to certain quantitative eye variables, but if the underlying genetic architecture does not support independent quantitative variation in these variables, the landscape is not smooth with respect to the actual genetic variation. This then leads directly to one of the core problems of evolvability theory, which is why the landscape is smooth in the first place (Kauffman, 1993). There are in fact two functions that need to be smooth, the mapping from phenotype to fitness (i.e. the adaptive landscape in the Simpsonian sense), and the mapping from genotype to phenotype. A core problem for a variational theory is thus to explain or predict the smoothness properties of the genotype–phenotype mapping.

Modularity is a simple way of reducing the ruggedness of fitness landscapes. Despite this, there are theoretical reasons to suspect that the simplest genetic architectures are not necessarily those that are most germane to evolvability. Conrad (1990) and Kauffman (1993) have provided several reasons to expect gene nets with intermediate connectivity to be the most favorable for evolvability. Starting with Fisher (1958), many authors have argued that an increase in phenotypic dimensionality favors evolvability by reducing the likelihood of being trapped in local optima (Conrad, 1990; Gordon, 1994; Gavrilets, 1997). From the perspective of a single character, integration with other aspects of the organism may provide for more opportunities to be perturbed from an inferior local optimum (Price et al., 1993).

To predict long-term evolvability we also need to understand the evolution of the variational properties themselves. This includes not only the direct changes in genetic variances and covariances, but more fundamentally, changes in mutational properties. With epistasis, both the genetic and the mutational variance matrices will be malleable, as the effects of both new and old alleles depend on the changing genetic background (Hansen and Wagner, 2001). If genes reinforce each other's effect in the direction of evolutionary change, variability will increase; if genes reduce or compensate each other, variability will decrease. Thus, sustained evolvability requires specific patterns of epistasis, and an understanding of gene interaction is therefore essential for understanding the evolution of variability and evolvability.

6. Discussion and conclusions

For Darwin the evolution of a complex eye was the ultimate challenge for the theory of natural selection. The tremendous developments and successes of selection theory in this century have largely answered this challenge. The amazing power and speed with which selection can build complex adaptations from available variation should no longer surprise us. The remaining puzzle is where the variations are coming from. Evolvability should not be taken for granted, it is something that needs to be explained, and this explanation must come from a theory of organismal variation.

A core assumption that facilitated long-term evolvability in the eye model was the implicit modular structure of the genotype–phenotype map. This is not an unusual assumption peculiar to this model, but reflects the conceptual structure of evolutionary biology as a whole, as the concept of a character refers to an individualized entity with independent evolutionary capabilities (Wagner, 1989b; Wagner and Laubichler, 2000). Thus, the very notion of a character presupposes a degree of modularity in the genotype–phenotype map.

Modularity may have come into focus in the study of evolvability because it is an easily operationalized property of the genotype–phenotype map. It has been almost universally accepted that biological organisms

are "modular", but the fact remains that pleiotropy across characters is a ubiquitous property of biological variation (see also Nagy and Williams (2001) for a recent challenge to the modularity paradigm). There is no agreement on how isolated a character must be to be counted as a module (Raff and Raff, 2000). It is therefore encouraging that explicit empirical assessments of modularity are becoming more common (e.g. Cheverud, 1996, 2001; Cheverud et al., 1997; Armbruster et al., 1999; Mezey et al., 2000; Raff and Sly, 2000; Klingenberg et al., 2001; Magwene, 2001; Hansen et al., 2003a). Most of these studies are based on comparing amount of pleiotropy or genetic correlation within characters with degree among characters. Modularity is a question of degree and can for example be quantified by counting the number of genes that have effects within a character versus the number that have pleiotropic effects across characters (Cheverud, 1996; Mezey et al., 2000).

The concept of conditional evolvability may be helpful in interpreting such studies, as it relates degree of modularity directly to evolvability. The field needs to reach some conclusions as to how much of the variability in a character really is useful for adaptation. This is particularly true for mutational variation. How much of the new mutational variation is available for adaptation in the sense that it is not compromised by deleterious side effects? I suggest this may be investigated by estimating mutational variances conditional on a measure of overall fitness.

A focus on the evolution of modularity may be too restrictive to produce a general understanding of evolvability. A possible implication of the results of this paper is that evolvability may be achieved in many different ways, and thus is not restricted to very special subsets of genetic architectures. This possibility needs further investigation, but if true it implies that the evolution of evolvability may not be as difficult as the evolution of modularity. An important aim of the field should be to identify and quantify genetic and developmental structures that facilitate or constrain evolvability. Variation in pleiotropic effects among genes is one structural feature that I suggest may facilitate evolvability. The pattern of epistasis and its relation to evolvability is another important area of research.

Modularity may refer to many different aspects of biological organization. In this essay I have focused on models that may most properly apply to morphological or life-history characters, but the dissociability of developmental processes is also important, as illustrated for example in the large literature on heterochrony (see, e.g. Gould (1977), and Raff (1996) for review). Several authors have also suggested that gene regulation is inherently modular in the sense that regulatory elements that control, say, the expression of the gene in a particular tissue may be added or deleted without introducing pleiotropic effects on the gene's expression in other tissues or circumstances (Dynan, 1989; Dickinson, 1990; Stern, 2000).

In the developmental genetics literature, the modularity concept is often applied to gene regulatory networks (Raff, 1996; Kirschner and Gerhart, 1998; Von Dassow and Munro, 1999). The idea is that sets of coregulated genes with specific functions may be co-opted and used as modules in a number of different circumstances. The segment–polarity system analyzed by Von Dassow et al. (2000) may serve as a paradigmatic example. This tightly regulated system is able to set up a stable spatial pattern of expression that is surprisingly robust to initial conditions and perturbations. In addition to its role in setting up the parasegments in the insect embryo, the system is also involved in a number of other circumstances including the patterning of appendages, and even setting up wing spots in butterflies. Homologues of the genes are also expressed in a number of tissues in vertebrates. It is easy to imagine that this robust system may have been co-opted many times as a ready-made pattern-forming module.

Although, it makes perfect sense to think of this as modularity from the developmental genetics point of view, it is not clear that it implies variational modularity on the morphological level. As many different genes in different systems are presumably regulated by members of the segment–polarity system, it may be difficult to co-opt and turn on the module in a novel setting without causing some deleterious mis-expression, and the recruitment may imply that many potential mutations in the regulation of the system will acquire new pleiotropic effects.

It is useful to contrast two extreme views on the meaning of co-option. The engineering view would be that the genetic system at large is so well regulated, hierarchical, and combinatorical that genes can easily be turned on or off in one set of circumstances without affecting the expression of the same or other

genes in different circumstances. Evolvability would then consist in the ability to recruit ready-made modules on all levels of organization into new combinations. In contrast, the tinkering view would be that co-option is associated with pleiotropic side effects and can only happen when the selective benefits are large. It may often be associated with the introduction of new pleiotropic effects and serve to make the genetic architecture more complex.

On the engineering view, understanding the evolution of evolvability seems like a hard problem, as it requires explaining the evolution of the engineering properties. The arguments in this essay suggest that an engineering perspective may not be necessary to assure evolvability. Complex genetic architectures may also be evolvable, even favor evolvability by introducing more variation in pleiotropic interactions, and adaptation may often have to pay the cost of a pleiotropic load.

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References

Armbruster, W.S., Di Stilio, V.S., Tuxill, J.D., Flores, T.C., Velásquez Runke, J.L., 1999. Covariance and decouping of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's, correlation-pleiades concept. Am. J. Bot. 86, 39–55.

Baatz, M., Wagner, G.P., 1997. Adaptive inertia caused by hidden pleiotropic effects. Theor. Pop. Biol. 51, 49–66.

Berg, R., 1960. The ecological significance of correlation pleiades. Evolution 17, 171–180.

Bonner, J.T., 1988. The Evolution of Complexity: by Means of Natural Selection. Princeton University Press, Princeton.

Bürger, R., 1986. Constraints for the evolution of functionally coupled characters. Evolution 40, 182–193.

Bürger, R., 2000. The Mathematical Theory of Selection, Recombination, and Mutation. Wiley, Chichester.

Cheverud, J.M., 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36, 44–50.

Cheverud, J.M., 2001. The genetic architecture of pleiotropic relations and differential epistasis. In: Wagner, G.P. (Ed.), The Character Concept in Evolutionary Biology. Academic press, San Diego, pp. 411–433.

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

Conrad, M., 1983. Adaptability: The Significance of Variability from Molecule to Ecosystem. Plenum press, New York.

Conrad, M., 1990. The geometry of evolution. BioSystems 24, 61–81.

Dawkins, R., 1996. Climbing Mount Improbable, Viking.

Dennett, D.C., 1995. Darwin's Dangerous Idea: Evolution and the Meanings of Life. Simon & Schuster, New York.

Dickinson, W.J., 1990. The evolution of regulatory genes and patterns in Drosophila. Evol. Biol. 25, 127–173.

Dynan, W.S., 1989. Modularity in promoters and enhancers. Cell 58, 1–4.

Fisher, R.A., 1958. The Genetical Theory of Natural Selection, 2nd ed. Dover, New York.

Gavrilets, S., 1997. Evolution and speciation on holey adaptive landscapes. TREE 12, 307–312.

Gerhart, J., Kirschner, M., 1997. Cells, Embryos and Evolution: Towards a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability. Blackwell Scientific Publications, Oxford.

Gordon, R., 1994. Evolution escapes rugged fitness landscapes by gene or genome doubling: the blessing of higher dimensionality. Computers Chem. 18, 325–331.

Gould, S.J., 1977. Ontogeny and Phylogeny. Belknap, Cambridge, MA.

Hansen, T.F., Wagner, G.P., 2001. Modeling genetic architecture: a multilinear model of gene interaction. Theor. Pop. Biol. 59, 61–86.

Hansen, T.F., Armbruster, W.S., Carlson, M.L., Pelabon, C., 2003a. Evolvability and genetic constraint in *Dalechampia* blossoms: Genetic correlations and conditional evolvability, J. Exper. Zool. (MDE) in press.

Hansen, T.F., Pelabon, C., Armbruster, W.S., Carlson, M.L., 2003b. Evolvability and genetic constraint in *Dalechampia* blossoms: Components of variance and measures of evolvability, J. Exper. Zool. (MDE) in press.

Hartl, D.L., Taubes, C.H., 1998. Towards a theory of evolutionary adaptation. Genetica 102–103, 525–533.

Hendry, A.P., Kinnison, M.T., 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution 53, 1637–1653.

Houle, D., 1998. How should we explain variation in the genetic variance of traits? Genetica 102/103, 241–253.

Houle, D., Morikawa, B., Lynch, M., 1996. Comparing mutational variabilities. Genetics 143, 1467–1483.

Jacob F., 1982. The Possible and the Actual. University of Washington press.

Kauffman, S.A., 1993. The Origins of Order. Self-Organization and Selection in Evolution, Oxford University Press, Oxford.

Kimura M., 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, UK.

Kirschner, M., Gerhart, J., 1998. Evolvability. Proc. Natl. Acad. Sci. 95, 8420–8427.

Klingenberg, C.P., Badyaev, A.V., Sowry, S.M., Beckwith, N.J., 2001. Inferring developmental modularity form morphological integration: analysis of individual variation and asymmetry in bumblebee wings. Am. Nat. 157, 11–23.

Lande, R., 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution 33, 402–416.

Lande, R., 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94, 203–215.

Lewontin, R.C., 1978. Adaptation. Sci. Am. 239, 212–231.

Lynch, M., 1988. The rate of polygenic mutation. Genet. Res. Camb. 51, 137–148.

Lynch, M., Blanchard, J., Houle, D., Kibota, T., Schultz, S., Vassilieva, L., Willis, J., 1999. Perspective: spontaneous deleterious mutation. Evolution 53, 645–663.

Magwene, P.M., 2001. New tools for studying integration and modularity. Evolution 55, 1734–1745.

Maynard Smith, J., Szathmáry, E., 1995. The Major Transitions in Evolution. Freeman, Oxford.

Mezey, J.G., Cheverud, J.M., Wagner, G.P., 2000. Is the genotype-phenotype map modular? A statistical approach using mouse quantitative trait loci. Genetics 156, 305–311.

Mitchell, W.A., Valone, T.J., 1990. The optimization research program: studying adaptations by their function. Quart. Rev. Biol. 65, 43–52.

Nagy, L.M., Williams, T.A., 2001. Comparative limb development as a tool for understanding the evolutionary diversification of limbs in arthropods: challenging the modularity paradigm. In: Wagner, G.P. (Ed.), The Character Concept in Evolutionary Biology. Academic Press, New York, pp. 455–488.

Needham, J., 1933. On the dissociability of the fundamental processes in ontogenesis. Biol. Rev. 8, 180–223.

Nilsson, D.E., Pelger, S., 1994. A pessimistic estimate of the time required for an eye to evolve. Proc. R. Soc. Lond. B256, 53–58.

Olson, E.C., Miller, R.L., 1958. Morphological Integration. The University of Chicago Press, Chicago.

Orr, H.A., 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52, 935–949.

Price, T., Turelli, M., Slatkin, M., 1993. Peak shifts produced by correlated response to selection. Evolution 47, 280–290.

Raff, R.A., 1996. The Shape of Life: Genes, Development, and the Evolution of Animal Form. University of Chicago Press, Chicago.

Raff, R.A., Sly, B.J., 2000. Modularity and dissociation in the evolution of gene expression territories in development. Evolution Develop. 2, 102–113.

Raff, E.C., Raff, R.A., 2000. Dissociability, modularity, evolvability. Evolution Develop. 2, 235–237.

Riedl, R.J., 1977. A systems-analytical approach to macro-evolutionary phenomena. Quart. Rev. Biol. 52, 351–370.

Riedl, R.J., 1978. Order in Living Organisms: a Systems Analysis of Evolution, Wiley, New York.

Rutherford, S.L., 2000. From genotype to phenotype: buffering mechanisms and the storage of genetic information. BioEssays 22, 1095–1105.

Stern, D., 2000. Evolutionary developmental biology and the problem of variation. Evolution 54, 1079–1091.

Turelli, M., 1985. Effects of pleiotropy on predictions concerning mutation-selection balance for polygenic traits. Genetics 111, 165–195.

Turelli, M., 1988. Population genetic models for polygenic variation and evolution. In: Weir, B.S., Eisen, E.J., Goodman, M.M., Namkoong, G. (Eds.), Proceedings of the Second International Conference on Quantitative Genetics, pp. 601–618.

Von Dassow, G., Munro, E.M., 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. J. Exp. Zool. (MDE) 285, 307–325.

Von Dassow, G., Meir, E., Munro, E.M., Odell, G.M., 2000. The segment polarity network is a robust developmental module. Nature 406, 188–192.

Wagner, G.P., 1984. Coevolution of functionally constrained characters: prerequisites of adaptive versatility. BioSystems 17, 51–55.

Wagner, G.P., 1988. The influence of variation and of developmental constraints on the rate of multivariate phenotypic evolution. J. Evol. Biol. 1, 45–66.

Wagner, G.P., 1989a. Multivariate mutation-selection balance with constrained pleiotropic effects. Genetics 122, 223–234.

Wagner, G.P., 1989b. The origin of morphological characters and the biological basis of homology. Evolution 43, 1157–1171.

Wagner, G.P., 1996. Homologues, natural kinds and the evolution of modularity. Am. Zool. 36, 36–43.

Wagner, G.P. (Ed). 2001. The character Concept in Evolutionary Biology. Academic Press, San Diego.

Wagner, G.P., Altenberg, L., 1996. Complex adaptations and evolution of evolvability. Evolution 50, 967–976.

Wagner, G.P., Laubichler, M.D., 2000. Character identification in evolutionary biology: the role of the organism. Theory Biosci. 119, 20–40 (Reprinted in Wagner GP. The character concept in evolutionary biology. Academic press, San Diego 2001).

Williams, G.C., 1992. Natural Selection: Domains, Levels, and Challenges. Oxford University Press, Oxford.

Wistow, G., 1993. Lens crystallins: gene recruitment and evolutionary dynamism. Trends Biochem. Sci. 18, 301–306.